Official Title: 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

NCT Number: NCT02576795

Applicant/MAH: BioMarin Pharmaceutical Inc.

Version Date: 24 Aug 2021

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI MD, PhD PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Subject Participation:	Approximately 368 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015
Date of Amendment 4:	02 September 2016
Date of Amendment 5:	14 February 2017
Date of Amendment 6:	21 December 2017
Date of Amendment 7:	10 October 2018
Date of Amendment 8:	31 January 2019
Date of Amendment 9:	19 June 2020
Date of Amendment 10:	24 August 2021

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 10

Date: 24 August 2021

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 10 to the 270-201 protocol is provided below.

1. Changes have been made to enhance screening for potential malignancies (including hepatic cancers) after dosing with BMN 270.

Rationale: The changes made include:

- Including a targeted liver ultrasound at the End of Year visits after Year 4 to screen for HCC (additional interim liver ultrasounds may be performed at the discretion of the Investigator);
- Recommending genomic analyses for any malignancy (except non-melanoma skin cancer) diagnosed during the course of the study.

Year-end liver ultrasounds are being implemented to assess the theoretical risk of HCC. While no cases of HCC have been reported in the Sponsor's AAV gene therapy non-clinical or clinical trials (more than 150 patients dosed, with some dosed more than 5 years ago), these assessments will further inform this theoretical risk.

2. Malignancy (except non-melanoma skin cancer) has been added as an Event of Special Interest (EOSI).

Rationale: The occurrence of malignancy (as above) was added as an EOSI for purposes of expedited safety reporting and additional safety monitoring.

3. Language has been added concerning the use of the SARS-CoV-2 vaccines.

Rationale: Due to the current worldwide SARS-CoV-2 pandemic and evolving availability and types of vaccines, language has been added to assist with timing and planning for vaccine administration. The Sponsor's recommendations reflect the risk assessment conducted on the currently available vaccines and guidance from multiple health agencies, and include information regarding different types of SARS-CoV-2 vaccines.

4. Guidance concerning the use of reactive corticosteroids for ALT elevations has been updated.

Rationale: The guidance suggests that subjects who are seeing ALT elevations more than 52 weeks after BMN 270 dosing should be started on reactive corticosteroids only after discussion between the Investigator and the Medical Monitor. Corticosteroids may be

delayed if elevations in ALT are clearly not related to BMN 270 (eg, elevated with concurrent increase in CPK due to intensive exercise).

5. The definition of treatment failure has been changed.

Rationale: The previous definition required either a failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 or an inability to maintain independence from prophylactic FVIII replacement therapy due to joint bleeding episodes; the revised definition requires both of these conditions to be present before a subject may be considered a treatment failure. The revision reflects the data seen as of the 52-week data cut for 270-301, where some subjects were still able to remain off of prophylactic FVIII replacement therapy (due to an absence of treated bleeding events) despite lower FVIII levels 1 or more years after BMN 270 infusion.

6. Frequency of several laboratory assessments after Year 1 has been decreased. These changes include:

   - Reducing FVIII Antigen BDD Assay to Q12W after Year 1 through Year 5, and Q26W in Years 6-7

     Rationale: Robust characterization is already available from Year 1 data, and more frequent sampling is not needed to understand long-term protein to activity ratios

   - Reducing AAV5 TAb to End of Year Visits only after Year 1

     Rationale: Antibody response is high and does not change much over time. Yearly testing should be sufficient to understand longer term responses, as little change is expected.

   - Reducing FVIII TAb to End of Year Visits only after Year 1

     Rationale: FVIII Bethesda Inhibitor is the main safety assessment. After 3 years, if there is a need in specific instances for data more frequent than once a year, backup plasma from another assay may be used for testing.

7. The identity of the Medical Monitor has been updated.
8. The summary of risks and benefits (Section 7) has been updated.
9. Minor administrative changes have been made for consistency and clarity.

Specific changes included in this amendment, including the Synopsis, since Amendment 9 (approved 19 June 2020) are outlined in Section 24.

2 SYNOPSIS

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TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain

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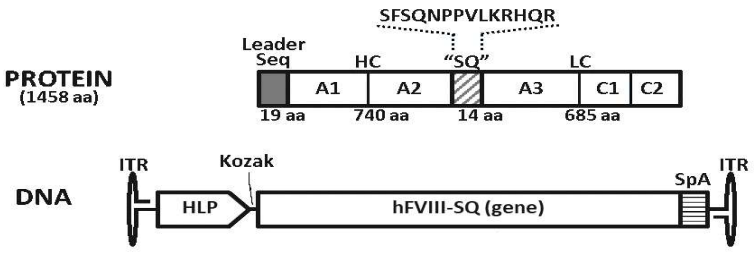
dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 1).

Figure 1. hFVIII-SQ Vector Genome and Encoded Protein



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Legend –Note that schematic is not to scale; aa = amino acids; ITR = inverted terminal repeat; HLP = human liver promoter; Kozak = Kozak concensus sequence (GCCACC); SpA = Synthetic poly(A) signal

BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

Liver Biopsy Substudy Rationale

The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms, and it is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans.

Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown.

The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to circularized genomes, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.

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OBJECTIVES:

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

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 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)

STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 7 years.

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Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)

Cohort 2: 2E13 vg per kilogram, iv

Cohort 3: 6E13 vg per kilogram, iv

Cohort 4: 4E13 vg per kilogram, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in non-human primates.

Cohorts 1-3

The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the one-stage clotting and chromogenic substrate assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

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For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥ 5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 and 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator. Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 7 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

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At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

Liver Biopsy Substudy Design

All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. Participation in the substudy may include liver biopsies at one or more time points. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy).

Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL).

NUMBER OF SUBJECTS PLANNED:

Up to 15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less

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2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive semen samples below the limit of detection of the test.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL

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9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria:

1. Currently enrolled in 270-201
2. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy
3. Able to sign informed consent and comply with requirements of the substudy
4. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy:

1. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to):
   - Significant thrombocytopenia (platelet count < 100 x 10^9/L)
   - Evidence of significant ascites

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- Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous infusion.

CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver tests (LTs, including ALT, AST, GGT, LDH, total bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52) during Year 1. LTs will be monitored every 4 weeks during Year 2, every 6 weeks for Years 3-5, and every 26 weeks for Years 6-7 post-dose in the safety extension; the frequency and duration of LT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

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Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage clotting and/or chromogenic substrate assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

Liver Biopsy Substudy:

The following exploratory assessments will be performed as part of the optional liver biopsy substudy:

- Morphologic/pathogenic changes after FVIII gene transduction or any change that may be associated with sustained ALT rise
- Determine quantities of liver FVIII-SQ DNA/RNA
- Determine forms of vector DNA in liver at the time of biopsy
- Determine percentage of hepatocytes expressing FVIII protein
- Determine percentage of hepatocytes staining positive for vector DNA
- Determine hepatic liver transcriptome at single nuclei level if sufficient material is obtained
- Identify the forms of vector DNA in the liver
- Examination of potential stress inducing cellular pathways
- Other exploratory assessment to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at $\geq$ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay at 16 weeks following study treatment. We can only assess the true steady state of

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FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates. Analysis of neutralizing antibody response and other immunological parameters as well as vector shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.		

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Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
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
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee
IND	Investigational New Drug (application)
INR	international normalized ratio
IP	investigational product
IV	intravenous
LT	liver test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the independent ethics committee (IEC) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IEC will be provided to BioMarin or its designee. The Investigator will provide the IEC with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IEC confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IEC and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IEC and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written informed consent before any study-related tests or evaluations are performed, as well as when updates to the ICF are made. Where it is not feasible for the Investigator to receive the signed, written ICF from the subject prior to beginning new or changed study-related procedures contained in such an ICF update (for example, due to COVID-19-related restrictions), the Investigator will ask the subject to verbally confirm during an informed consent interview that the subject has signed and dated the ICF. The Investigator should document this verbal confirmation on the Investigator's copy of the ICF and, when it is possible to do so, receive the informed consent signed by the subject and archive the original(s) in the record file of the subject.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IEC approval. BioMarin and the IEC must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the

approved subject information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver tests (LTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Iorio 2019). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp 2012) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker 2010). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman 2013). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay 2012).

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava 2020); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD),

pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in the nonclinical and clinical programs. Though not observed in the nonclinical species, liver

toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani 2006). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda 2013).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous and Ongoing Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno 2003) and an AAV8 vector (Nathwani 2011; Nathwani 2014). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh 2013).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola 2014). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

Ongoing clinical studies for BMN 270 include:

- 270-203, a phase 2 study in patients with severe HA who have anti-AAV5 antibody titers
- 270-205, a phase 1/2 study in patients with severe HA who have active or prior FVIII inhibitors
- 270-301, the pivotal phase 3 study in patients with severe HA who receive BMN 270 at the 6E13 vg/kg dose level
- 270-302, a phase 3 study in patients with severe HA who receive BMN 270 at the 4E13 vg/kg dose level
- 270-303, a phase 3 study in patients with severe HA who received BMN 270 at the 6E13 vg/kg dose level along with prophylactic corticosteroids

A comprehensive review of safety, efficacy, and immunogenicity results as of the latest data cut is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Iorio 2019). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel 2011). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and

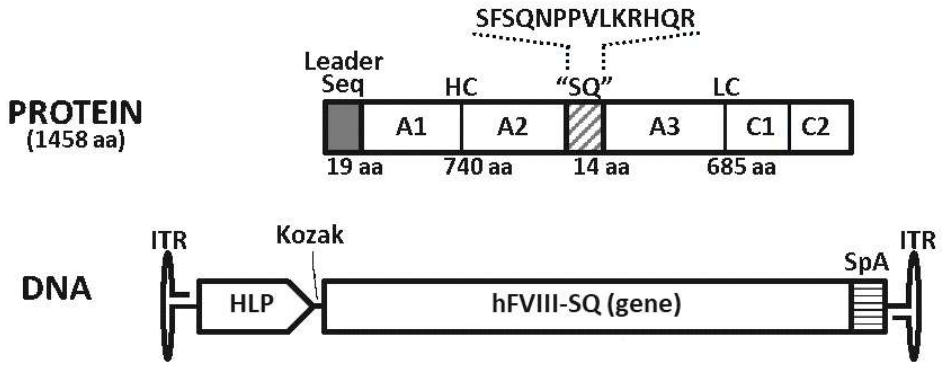
substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker 2010; Mahlangu 2014). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci 2001). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani 2005) for serotypes 2, 5 and 8 among others. Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani 2014). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani 2014).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: hFVIII-SQ Vector Genome and Encoded Protein



Legend –Note that schematic is not to scale; aa = amino acids; ITR = inverted terminal repeat; HLP = human liver promoter; Kozak = Kozak concensus sequence (GCCACC); SpA = Synthetic poly(A) signal

BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.3.1 Liver Biopsy Substudy Rationale

The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms. It is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans.

Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as

inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or the variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown. Finally, a call to incorporate liver biopsy substudies into gene therapy trials for hemophilia has been issued by medical and scientific leaders in the field to help illuminate these and other questions (National Hemophilia Foundation 2019).

The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to genome circularization, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.

7.4 Summary of Overall Risks and Benefits

BMN 270 has an acceptable safety and tolerability profile that supports a positive benefit-risk assessment. Single infusions have been generally well tolerated by treated subjects across all investigated doses. All subjects have successfully completed their full-dose infusion of BMN 270, with no infusions requiring permanent termination prior to completion due to AEs. No deaths have been reported in any of the BMN 270 studies, and no participants discontinued from studies as a result of an AE. Frequency of adverse events decreased over time with no delayed adverse drug reactions.

Infusion reactions associated with BMN 270 administration included symptoms such as maculopapular rash, urticaria, nausea, diarrhea, watery eyes, rigors, chills, myalgia, fever, tachycardia and hypotension emerging within 24 hours of receiving BMN 270. All of these events subsided without clinical sequela within 48 hours following medical management Infusion-related reactions were effectively mitigated by managing infusion rate and medications.

Transient, asymptomatic ALT elevation (grade 1 to 3 in severity) was observed in most subjects administered BMN 270 shortly after dosing, with no symptoms or sequelae suggestive of clinically significant hepatocyte injury or liver dysfunction. Across the 6E13 vg/kg cohort of 270-201 and 270-301, subjects enrolled in 270-201 developed ALT elevation about 5.5 weeks later than subjects 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 seen between the 6E13 vg/kg subjects in 270-201 and the subjects in 270-301 could be attributed to the difference in the protocol-specified corticosteroid regimens in place in those studies,

including the early use of corticosteroids (ie, by Week 3 post-BMN 270 infusion). While the majority of ALT elevations responded rapidly to corticosteroids, given current interest in the field of AAV gene therapy for the use of non-steroidal approaches to managing or preventing ALT elevations, alternate non-steroidal systemic immunosuppressive agents have also been used to manage hepatic reactions where corticosteroids have proven to be ineffective or where high doses/and or prolonged exposure to corticosteroids have led to unwanted side effects. Overall, the literature and clinical experience with BMN 270 suggests that transient elevations in liver enzymes are expected following AAV-based gene therapy for the treatment for hemophilia A or B without any long-term concerns of hepatic injury (Manno 2006; Nathwani 2011; George 2016; Miesbach 2016; Pasi 2020).

At the highest dose tested in 270-201 (6E13 vg/kg), the majority of subjects achieved FVIII levels above 50 IU/dL at 52 weeks post-infusion. Subjects in that cohort also reported markedly decreased bleeding compared with pre-study rates and the ability to discontinue prophylactic FVIII infusions. Subjects at all dose levels continue to be followed.

In 270-301, an interim analysis has shown increased FVIII activity in the majority of subjects to mild HA or normal levels at 26 weeks post-infusion, also with markedly decreased bleeding compared with pre-study rates and the ability to discontinue prophylactic FVIII infusions. All subjects who were included in the 1-year analysis have been dosed with 6E13 vg/kg and continue to be followed.

The current data available has shown an established positive benefit-risk profile for BMN 270 at the 6E13 vg/kg dosing level. Given the monitoring measures in place in the clinical protocol(s) to minimize the risk to subjects participating in the existing studies, the identified risks are justified by the anticipated benefits that may be afforded to subjects. Each subject in 270-301 will have a comprehensive surveillance plan that monitors LTs during the study, and elevations in LTs will be addressed according to the guidelines set forth in the protocol. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

For additional information on safety findings from clinical studies, refer to current version of the Investigator's Brochure.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon

the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

7.4.1 Optional Liver Biopsy Substudy Risks and Benefits

Liver biopsy is considered a safe procedure, with serious complications occurring less than once in every 10,000 procedures (Grant 2004). Although the theoretical risks of significant complications are extremely small, the main complications would include bleeding and bile leakage. Another theoretical complication is infection at the needle insertion site; the sterile technique used makes this risk extremely small.

The most common problems include mild pain and a minor decrease in blood pressure. More serious complications, such as bleeding, infection, and injury to nearby organs, are very rare, but the subject will be monitored appropriately to ensure correct management should any of these occur. Any complications related to the liver biopsy should be reported as adverse events, as outlined in Section 10. The liver biopsy is a standard investigation, and will be explained more fully by the experienced clinician performing the biopsy.

Each subject who participates in this optional substudy will have a comprehensive pre-/post-biopsy surveillance plan according to the standard procedures at the institution. Safety will be assessed by adverse event reporting and clinical laboratory assessments. Per the Investigator's discretion and/or according to local guidelines, the subject may be kept in overnight following the liver biopsy for additional safety monitoring; such an overnight stay would not be considered a hospitalization for serious adverse event (SAE) reporting purposes (refer to Section 10.4.1.7).

There is no direct benefit from participating in this study other than contributing to understanding the mechanism of action of BMN 270. Consenting into this specific substudy is optional and will not have any effect on the subject’s continued participation in 270-201.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

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 rAAV genomic DNA (eg, concatemers) are present at the time of biopsy
- To determine the transduction pattern in humans (ie, peri-portal hepatocytes, central vein hepatocytes)

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 7 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

- Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
- Cohort 2: 2E13 vg per kilogram, iv
- Cohort 3: 6E13 vg per kilogram, iv
- Cohort 4: 4E13 vg per kilogram, iv

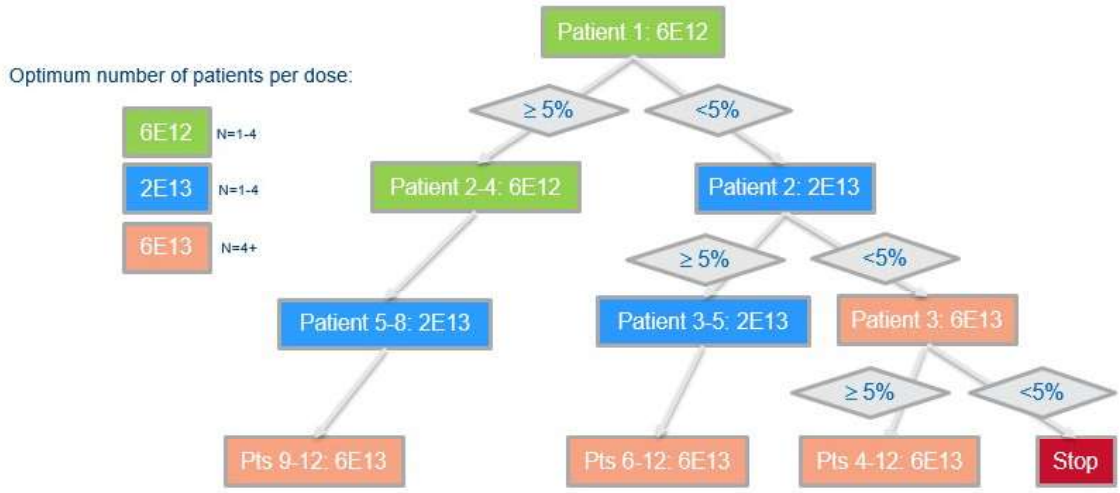
The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in mice.

Cohorts 1-3

The first three cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches ≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme for Cohorts 1 to 3



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the Data Review Board (DRB). The adaptive nature of this trial allows the DRB

to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 and 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 7 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected PBMC. Additionally, if any of the events listed in Section 9.3.4.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the DRB and the Sponsor, it is deemed

appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3, Table 9.1.4, and Table 9.1.5 for Safety Follow-up.

Table 9.1.6, dealing with reactive corticosteroid use in the event of elevated LTs, is discussed in Section 9.4.8.2.

Liver Biopsy Substudy Design

All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy).

Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL).

Table 9.1.7 presents the schedule of assessments for participation in the liver biopsy substudy.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	Smart Rescreening[i] (Day -28 to Day -1)	Baseline (Day -7 to Day -1)[h]	
Informed consent	X			
Medical History	X			
Physical Examination[a]	X		X	X
Height and Weight[a]	X			
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, coagulation screen, and CRP[e]	X	X	X	
Urine Tests[e]	X	X	X	
Liver Tests[e]	X	X	X	
PBMC collection for CTL baseline			X	
Von Willebrand Factor Antigen (VWF:Ag)	X			
Direct Thrombin Test			X	
PCR of vector DNA in blood, saliva, urine, semen, and stools			X	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

[b] Includes baseline hFVIII activity (chromogenic substrate assay and one-stage clotting assay), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). Sample collection on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion must be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up (Week 1-16)

Assessment	Follow-Up After BMN 270 Administration – Weeks*																
	Week 1																
	D2	D4	D8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical examination[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X		X				X
Urine Tests[b]						X				X				X				X
Liver Tests (local)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer						X				X				X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X	X	X			X		X		X		X		X		X		X
Exploratory biomarker assessments[f]						X				X				X				X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C																		X[g]
PBMC collection			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Von Willebrand Factor Antigen (VWF:Ag)						X				X				X				X
Direct Thrombin test			X												X			

* Visit windows are ± 48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.9.5.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then until at least 3 consecutive negative results are obtained. Collection and testing of semen samples must continue through Week 12, even if 3 consecutive negative samples in that compartment have already been recorded.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[g] Testing for reactivation of hepatitis B and hepatitis C at Week 16, for subjects with a past medical history of Hepatitis B or Hepatitis C prior to study entry, should be performed only in subjects who have not received reactive corticosteroids prior to Week 16; subjects who have received reactive corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.6.

Table 9.1.3: Schedule of Events – Safety Follow-Up (Week 17-32)

Assessment	Follow-Up After BMN 270 Administration – Weeks*															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight										X						
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X				X				X				X
Urine Tests[b]				X				X				X				X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer				X								X				
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X				X				X				X
Exploratory biomarker assessments[f]				X				X				X				X
Haemo-QoL-A QoL assessment												X				
AAV5 antibody titer				X				X				X				X
PBMC collection	X	X	X	X		X		X		X		X		X		X
Von Willebrand Factor Antigen (VWF:Ag)				X				X				X				X
Direct Thrombin test										X						

* Visit windows are ± 48 hours

[a] Brief physical examination should be done at all weekly visits. Refer to Section 9.7.9.5.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection for each matrix to occur until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.4: Schedule of Events – Safety Follow-Up (Week 33-52)

	Year 1 – Weeks*											
Assessment	**33**	**34**	**35**	**36**	**38**	**40**	**42**	**44**	**46**	**48**	**50**	**52**
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X
Weight[a]												X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X
Urine Tests[b]				X		X		X		X		X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X
AAV5 antibody titer				X		X		X		X		X
FVIII antibody titer				X				X				X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)		X		X				X				X
Von Willebrand Factor Antigen (VWF:Ag)				X		X		X		X		X
Direct Thrombin Test					X							X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X		X		X		X		X
Exploratory biomarker assessments[f]				X		X		X		X		X
Haemo-QoL-A QoL assessment												X

* Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52.

[a] Complete physical examination should be performed at Week 52; brief physical examination may be performed at other study visits. Refer to Section 9.7.9.5.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least

twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.5: Schedule of Events – Safety Follow-Up (Years 2-7)

Assessment	Years 2-5*	Year 2*	Years 3-5*	Years 6-7		End of Year Visits						
						Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	
	Q12W	Q4W[h]	Q6W[h,j]	Q13W (Remote)[i]	Q26W	W104	W156	W208	W260	W312	W364	ETV
Physical examination[a]	X[a]					X[a]				X	X	X
Weight[a]	X[a]					X[a]				X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X				X	X	X
Vital Signs	X					X				X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]	X				X	X				X	X	X
Urine Tests[b]	X					X						X
Liver Tests (local)[b]	X	X	X			X						X
FVIII assays (local)[c]	X	X	X			X						X
Liver Tests (central)[b]	X	X	X		X	X				X	X	X
FVIII assays (central)[d]	X	X	X		X	X				X	X	X
FVIII Antigen assay	X				X	X				X	X	X
AAV5 antibody titer						X				X	X	X
FVIII antibody titer						X				X	X	X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)	X					X						X
Von Willebrand Factor Antigen (VWF:Ag)	X					X						X
PCR of vector DNA in semen[e]	X[e]	X[e]	X[e]		X[e]	X[e]				X[e]	X[e]	X
PRC of vector DNA in blood, saliva, urine, stools[e]	X[e]				X[e]	X[e]				X[e]	X[e]	X
Exploratory biomarker assessments[g]	X					X				X	X	X

Assessment	Years 2-5*	Year 2*	Years 3-5*	Years 6-7		End of Year Visits						
						Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	
	Q12W	Q4W[h]	Q6W[h,j]	Q13W (Remote)[i]	Q26W	W104	W156	W208	W260	W312	W364	ETV
Haemo-QoL-A QoL assessment	X[f]				X[f]	X[f]				X[f]	X[f]	X
Liver ultrasound[k]						X[k] (Year 5 only)				X[k]	X[k]	X

ETV: Early Termination Visit

[*] Visit windows are ± 2 weeks for visits in Years 2-5 and ± 4 weeks for visits in Years 6-7. The Q6W visits during Years 3-5 should restart after each End of Year visit (eg, the first Q6W visit during Year 3 should be ~6 weeks after the End of Year 2 visit).

[a] Complete physical examination should be performed at the End of Year visits (genitourinary examination may be deferred unless the subject has genitourinary-related complaints); brief physical examination may be performed at other study visits. Weight should be recorded at the second Q12W visit each year during Years 2-5, and at every End of Year visit during Years 2-7.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be assessed more frequently when ALT values are ≥1.5x ULN or based upon discussion between the Medical Monitor and the Investigator. Subjects with ALT ≥ 1.5x ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment;; or (2) after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. During Years 2-5 of the Post-Infusion Follow-Up period, urine tests and blood, chemistry, and coagulation tests should be performed at the second Q12W visit each year and at every End of Year visit. During Years 6-7, these tests should be performed at the Q26W and End of Year visits.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) during Years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, and hFVIII coagulation activity exploratory assay. If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity

measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) in Years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), and/or every 26 weeks (during Years 6-7) until 3 consecutive samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).

[f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at the second Q12W visit each year and at every End of Year visit. During Years 6-7, the Haemo-QoL-A assessment should be performed at the Q26W visits and the End of Year visits.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Subjects who meet the definition of treatment failure to BMN 270 therapy after Week 52 may omit the Q4W and Q6W visits during Years 2-5, and must attend only the Q12W and End of Year visits. Such subjects following the abbreviated schedule who have not yet cleared vector shedding in semen must still provide samples Q4W (during Year 2), Q6W (during Years 3-5), or Q26W (during Years 6-7), until vector shedding has been cleared (but do not need to do other scheduled assessments at those visit dates), either by reporting to the site to provide samples or by providing those samples to an MN professional.

[i] For every 13 week remote visits during Years 6-7, the site should contact the subject to collect data on adverse events, new or changed concomitant medications, and diary entries (FVIII usage and bleeding events).

[j] At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office. Refer to Section 9.7.9.2 for details.

[k] Additional liver ultrasounds may be performed at interim timepoints (ie, between the End of Year visits) at the discretion of the Investigator.

Table 9.1.6: Schedule of Events – Reactive Corticosteroids for LT Elevations

	Steroid Treatment Period[d]											Post-Steroid Period[c]				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11[b]**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 13**
Reactive corticosteroids (dose in mg/day) [a]	60 mg	60 mg	40 mg	40 mg	30 mg	30 mg	20 mg	20 mg	15 mg	10 mg	5 mg					
FVIII activity testing												X	X	X	X	
Liver testing												X	X	X	X	
Hepatitis B testing[e]						X						X				X
HCV Viral Load[e]						X						X				X

[a] Reactive corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor.

[b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration timing of the ALT elevation (after Week 52), as well as possible confounders for the ALT elevation and adverse events related to corticosteroid dosing. Guidance for tapering oral corticosteroids can be found in Section 9.4.8.2.

[c] After discontinuation of corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. If these assessments are already being done as part of normal study follow-up, they do not need to be duplicated.

[d] Regardless of the assessments listed in the Schedule of Assessments (Table 9.1.2, Table 9.1.3, or Table 9.1.4), subjects initiated on corticosteroids will only be required to have laboratory evaluations on a weekly basis.

[e] Should only be performed in subjects with a history of hepatitis B or hepatitis C prior to study entry.

Table 9.1.7: Schedule of Events – Liver Biopsy Substudy

	Within 28 Days Before Biopsy Day	Within 7 Days Before Biopsy Day	1 Day Before Biopsy Day [d]	Biopsy Day (BD)
Informed Consent	X			
Liver Ultrasound[a]	X			
Brief Physical Examination	X		X	X
Hematology, Coagulation, Chemistry Assessments[b]	X		X	
Liver Tests[b]	X		X	X
FibroScan		X		
Local FVIII Activity Level Assessment		X	X	X
Pre-Biopsy Consultation[c]		X		
Liver Biopsy[e]				X

[a] Liver ultrasound must be performed within 28 days prior to the scheduled biopsy, unless an ultrasound result from the prior 3 months is already available.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests.

[c] Subjects will undergo a pre-biopsy consultation with the investigator (treating hematologist).

[d] Subjects may need to repeat/extend this visit if their FVIII activity levels need adjustment before the biopsy, at the discretion of the investigator (treating hematologist).

[e] Biopsy will be a percutaneous or transjugular biopsy under ultrasound guidance, performed according to the standard procedure of the institution. If only a small amount of tissue (< 2 cm) is obtained at the time of the biopsy, the subject may be asked to consent for a second pass. In this case, the original < 2 cm sample should still be retained and handled according to the instructions for handling biopsy specimens in the Laboratory Manual. Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive semen samples below the limit of detection of the test.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4.
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Liver Biopsy Substudy Inclusion and Exclusion Criteria

Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria:

1. Currently enrolled in 270-201
2. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy
3. Able to sign informed consent and comply with requirements of the substudy
4. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy:

1. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to):
   - Significant thrombocytopenia (platelet count < 100 x 10^9/L)
   - Evidence of significant ascites
   - Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.

9.3.4 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

Subjects may be considered lost to follow-up if the site has documented at least 4 attempted contacts by key research personnel to reach the subject without success in the following manner:

- 2 attempts by telephone or email (if possible); then
- If telephone/email contacts are unsuccessful, 2 attempts must be made by certified letter or by appropriate local process.

Where communication has been made by phone, this should be documented in the subject source notes.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IEC. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- Any ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270, in the absence of a definitive alternate etiology for the increase

- The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion
- The detection of AAV vector DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than 52 weeks after BMN 270 infusion, as discussed in Section 9.7.9.7
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt:

- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days

9.3.5 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.6 Duration of Subject Participation

The duration of this study will be approximately 368 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 348 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical examination performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (eg, the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be discharged from the clinic unless toxicity has been observed in which case the stay in the clinic may be

extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

For Cohorts 1 to 3, approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in that cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design for Cohorts 1-3

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents
- Emicizumab

- Fitusiran
- Concizumab
- Efavirenz

Subjects who begin antiretroviral therapy following BMN 270 infusion and during their participation in 270-201 must have their antiretroviral medication regimen approved by the Investigator and Medical Monitor prior to starting antiretroviral treatment.

Medications which are predominately metabolized by the liver (eg, acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.

Administration of SARS-CoV-2 vaccine after BMN 270 infusion may occur after consultation between Investigator and Medical Monitor. Investigators should use clinical judgment, taking into consideration local factors, individual risk factors, and the benefit/risk related to timing of vaccine administration.

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "episodic" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "episodic" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52). LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

9.4.8.2.1 Reactive Corticosteroids

In general, reactive corticosteroids may be initiated when a subject's ALT value is $\geq$ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator. Corticosteroids may be delayed, upon discussion with the Medical Monitor, if elevations in ALT are clearly not related to BMN 270 (eg, elevated ALT with concurrent increase in CPK due to intensive exercise) and in particular for elevations occurring more than 52 weeks after BMN 270 dosing.

Reports of raised LTs (defined as ALT ≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

Following initiation or completion of reactive corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

Unless otherwise indicated, treatment with reactive corticosteroids should be initiated at a dose of 60 mg per day and then gradually tapered if the ALT level remains stable or declines after 2 weeks (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.6).

Should a scenario arise in which differences from the minimum recommended dose and/or duration of reactive corticosteroids may be clinically indicated, a discussion should take place between the Investigator and Medical Monitor regarding corticosteroid dose adjustments. Management of ALT elevations with reactive corticosteroids, including tapering of doses and managing worsening and/or recurrent ALT elevations, should be guided by the following (Table 9.4.8.2.1.1):

Table 9.4.8.2.1.1: Management of ALT Elevations with Reactive Corticosteroids

ALT ≥ 1.5x Baseline or > ULN	• Repeat LTs and FVIII within 24-72 hours • Continue to monitor LTs until ALT is stable or not increasing • Investigate for alternative etiologies (eg, concomitant medications, viral or autoimmune hepatitis, alcohol use, recreational drug use, special diets, strenuous exercise, prior and/or concurrent illnesses, exposure to environmental and/or industrial chemicals, etc.) • If no alternative etiology is found, contact the Medical Monitor to discuss the initiation of reactive corticosteroids with the following tapering schedule: 60 mg x 2 weeks; 40 mg x 3 weeks; 30 mg x 1 week; 20 mg x 1 week; 10 mg x 1 week upon consultation with the Medical Monitor • Consider evaluation with additional liver tests (including but not limited to ALT, AST, bilirubin, and alkaline phosphatase) • Consider obtaining other possibly relevant laboratory evaluations (albumin, PT/INR, CRP, etc.) • Consider obtaining complete blood count with differential to assess for eosinophilia • Consider obtaining PBMC, C3, C3a, Bb, and sC5b-9 to evaluate potential immune response (prior to starting reactive oral corticosteroids) • Continue to taper as long as subject's ALT is not increasing. Decisions regarding regimen modification may be made based upon Investigator judgement and discussion with the Medical Monitor • For any ALT elevations that begin after 52 weeks on study, please consult the Medical Monitor prior to initiating corticosteroids unless there is an imminent safety concern
Worsening ALT	If after 2 weeks ALT levels have worsened with corticosteroid dose of 60 mg/day, the following is recommended: • Investigate for alternative etiologies including labs noted above, if not previously checked • Increase corticosteroid dose up to a maximum of 1.2 mg/kg for no more than 2 weeks • For subjects who are refractory to the maximum dose of corticosteroids, or intolerant to use of corticosteroids, consider use of alternative immunosuppressants (tacrolimus or mycophenolate) • Consider gastroenterology and/or hepatology consult, abdominal workup, imaging (including MRI or ultrasound), and/or liver biopsy as appropriate Any concerns should be discussed between the Investigator and the Medical Monitor
Recurrent ALT elevations	If the subject has recurrent ALT elevations (≥ 1.5x Baseline or > ULN) and there are no safety concerns, the decision regarding management may be made at the discretion of the Investigator after discussion 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.

When ruling out alternative viral or autoimmune hepatitis as part of the elevated ALT workup, the following tests should be performed (Table 9.4.8.2.1.2):

Table 9.4.8.2.1.2: Viral and Autoimmune Hepatitis Testing

Viral Hepatitis Workup Testing	Autoimmune Hepatitis Workup Testing
Hepatitis A	Smooth muscle antibody
Hepatitis B	Mitochondrial antibody
Hepatitis C	Liver/kidney microsomal antibodies
Hepatitis E	Antinuclear antibody (ANA) HEP-2
Cytomegalovirus (CMV)	
Epstein-Barr virus (EBV)	
Herpes simplex virus (HSV) 1 & 2	

After discontinuation of reactive corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values.

Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. The use of COX-2 inhibitors, while not contraindicated during corticosteroid treatment, should be limited, if possible. Practical management to prevent complications related to oral corticosteroid therapy may be undertaken at the discretion of the Investigator (eg, evaluation of glucose intolerance, hyperlipidemia etc.). For the subjects with a past medical history of hepatitis B or hepatitis C prior to study entry, Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

Subjects on corticosteroids should receive appropriate counselling and support regarding side effects from the Investigator or the treating institution (eg, listings of side effects and when to notify carers, wallet card for emergencies if on steroids, etc.). Additional management, including the co-prescription of additional medications to prevent complications related to corticosteroid therapy, may be undertaken at the discretion of the investigator, including, but not limited to, prophylaxis against the occurrence of gastric ulcers, osteoporosis, and infections.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on a dispensing log. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data, if allowed by local SOPs.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials (or must be referenced in their institution SOPs).

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a study drug return form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

Subjects should be advised to abstain from any blood or sperm donation after BMN 270 infusion, until there is no further evidence of vector shedding from PCR analysis of samples.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.9.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1 through Table 9.1.5) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to expression of FVIII at 5 IU/dL or above, (ie, a mild severity). This is associated in natural history studies with clinically superior long term outcomes (Den Ujil 2011).

The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic substrate assay)
- FVIII activity by one-stage clotting assay

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII $\geq$ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

The FVIII activity level in both assays and the number of subjects with FVIII activity $\geq$ 5 IU/dL in at least one of the two assays will be summarized.

FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5 but will be used in conjunction with local lab LT assessments to monitor subject safety and need for initiation of reactive corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as failure to achieve FVIII activity $\geq$ 5 IU/dL by Week 52 and 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage clotting and/or chromogenic substrate assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5.

9.7.6 Exploratory Assessments

Blood samples will be collected at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

Liver biopsy samples collected as part of the exploratory substudy may also be tested to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer. Subjects participating in the substudy may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed.

On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with samples collected specifically for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Liver Biopsy Substudy

The objectives of the liver biopsy substudy are considered exploratory.

Subjects who consent to the procedure will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound exam of the liver within 28 days prior to the procedure (to ensure there is no obstruction to the liver that would interfere with the liver biopsy) and a FibroScan of the liver within one week before the liver

biopsy (to correlate any histopathologic findings on the biopsy). Subjects will also undergo a pre-biopsy consultation with the investigator (treating hematologist).

Within 24 hours prior to the biopsy being performed, subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). FVIII activity levels for this purpose should be assessed at the local laboratory within 7 days before the biopsy, and again the day before the biopsy. As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".

The liver biopsy should be performed in the morning, and the biopsy procedure and follow-up care should be done according to the local standard of care. Additional details for handling the biopsy specimens are provided in the Laboratory Manual.

Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion and/or according to local guidelines.

Participation in the substudy may include liver biopsies at one or more time points.

Safety findings arising from the histopathological analysis of the biopsy sample are subject to Adverse Event reporting (Section 10), and such findings should be further assessed and followed-up as clinically appropriate and in order to safeguard the subject's ongoing medical care (this should be done in consultation with a hepatologist and/or other specialist clinicians if required). In the event fibrotic changes being observed on the biopsy sample, additional follow-up Fibroscans may be considered (with the frequency of subsequent scans at the discretion of the Investigator and/or hepatologist).

Where a biopsy has been taken for safety-related reasons (rather than as part of the liver biopsy substudy) or was available from a past procedure, the Sponsor may want to see that biopsy information to help evaluate the impact of BMN 270 on the liver. The Sponsor may request that slides from a liver biopsy not performed as part of the substudy be made available for additional histopathological review.

9.7.9 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.9.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5.

9.7.9.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. The scheduled clinical laboratory tests are listed in Table 9.7.9.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.9.2.1: Clinical Laboratory Tests

Blood Chemistry	Haematology	Urine Tests	Other
Albumin	Haemoglobin	Appearance	CRP
BUN	Haematocrit	Color	
Calcium	WBC count	pH	**Coagulation Screen including:**
Chloride	RBC count	Specific gravity	
Total cholesterol	Platelet count	Ketones	APTT
CO_2	Differential cell count	Protein	PT/INR
CPK		Glucose	TT
Creatinine		Bilirubin	
Glucose		Nitrite	**Other Tests:**
Phosphorus		Urobilinogen	ABO blood typing*
Potassium		Haemoglobin	
Total protein			
Sodium			
Uric Acid			

BUN, blood urea nitrogen; CO_2, carbon dioxide; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

* ABO blood typing assessment should be completed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study).

At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

9.7.9.3 Malignancies

Liver ultrasounds will be performed annually at each End of Year visit starting at Year 5 (Week 260) through the end of the study to screen for HCC. Additional liver ultrasounds may be performed between the End of Year visits at the discretion of the Investigator.

Any development of a malignancy (except non-melanoma skin cancers) during the course of the study will be considered an EOSI (refer to Section 10.2.1) and is subject to expedited reporting. In addition, it is recommended that genomic analyses be performed on any malignancy (except non-melanoma skin cancers) diagnosed during the course of the study. The study site will coordinate sending samples from the malignancy for genomic analyses, if available.

9.7.9.4 Liver and Hepatitis Testing

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active hepatitis B or hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing hepatitis B or hepatitis C infection is exclusionary. Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received as part of their medical history assessment at Screening.

Subjects with a history of hepatitis B or hepatitis C infection prior to study entry will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive reactive corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

A liver ultrasound and LTs at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

Liver tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. At each time point, the following LTs should be assessed:

Table 9.7.9.3.1: Liver Tests

Liver Tests (LTs)			
Alkaline Phosphatase	AST (SGOT)	Total Bilirubin	LDH
ALT (SGPT)	Direct Bilirubin	GGT	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase

Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

9.7.9.5 HIV Testing

HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.

9.7.9.6 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and at the End of Years visits thereafter. A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems. The genitourinary examination may be deferred for visits after Year 1 unless the subject has genitourinary-related complaints.

At other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, dermatologic,

musculoskeletal, and gastrointestinal assessments. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

Height will be recorded at Screening only. Weight will be recorded at Screening, at Weeks 26 and 52 during Year 1, and then at the second Q12W visit each year during Years 2-5 and at every End of Year visit during Years 2-7.

9.7.9.7 Vector Shedding

Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani 2014; Manno 2006; Schenk-Braat 2007; Croteau 2004). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid but not in motile sperm (Manno 2006). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach 2016).

During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of vector shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), and every 26 weeks (during Years 6-7) until 3 consecutive semen samples below the limit of detection of the test are documented (or upon consultation between the Investigator and Medical Monitor). Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule (refer to Section 12.5.3 but who have not cleared vector shedding from semen must still provide samples for assessment until vector shedding has cleared:

every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), or every 26 weeks (during Years 6-7), either by reporting to the site to provide samples or by providing those samples to an MN professional.

Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive semen samples below the limit of detection (upon consultation between the Investigator and Medical Monitor).

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (eg, sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.1.1.1 Bleeding and Suspected Bleeding Events

All bleeding events and suspected bleeding events, regardless of the need for exogenous FVIII therapy as treatment, should be captured in subject diaries and recorded on the designated bleeding eCRF. Bleeding events and suspected bleeding events should not be reported as adverse events, with the following exception:

- All bleeding events and suspected bleeding events which meet one or more of the criteria for being serious (refer to Section 10.2) should be reported as serious adverse events (whether or not they are bleeding events that are normal sequelae of hemophilia, and whether or not they required exogenous FVIII as treatment).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening
- *Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.*
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (eg, anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of ALT ≥ 1.5x ULN, regardless of whether that triggers an initiation or modification of corticosteroid treatment
- Events meeting the criteria for Hy's law (ALT or AST elevation ≥ 3x ULN plus total bilirubin ≥ 2x ULN)
- Thromboembolic event
- Development of anti-FVIII inhibitory antibodies (inhibitors)
- Any new diagnosis of malignancy (except non-melanoma skin cancer)

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 7 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.1.1.1.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.1.1.1 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.1.1.1. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against

cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on an eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE (or SAE, as appropriate) eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. Events that change in severity necessitate the recording of an additional AE. AEs that do not have a change in severity should be recorded only once on the eCRF.

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE or EOSI should be reported as such, and recorded in the AE eCRF. Any laboratory result abnormality of CTCAE Grade 4 or 5 should be recorded as an SAE in the AE eCRF, unless the abnormal laboratory results has been reported or captured as part of an underlying diagnosis.

A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Requiring a change in concomitant therapy (eg, addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless there is an impact to clinical outcomes (eg, increased rate of bleeding, worsening of joint disease).

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.1.1.1).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated procedure or assessment
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Insert an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug or FVIII replacement therapy.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record “Unexplained Death” or “Death of Unknown Cause” on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the

Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up eCRF. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IEC is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any

events which may require the implementation of urgent safety measures to BioMarin within 24 hours of becoming aware of the event.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax: PI
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI, MD, PhD
Address: Biomarin Pharmaceutical Inc.
105 Digital Drive
Novato, CA 94949
Phone PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic substrate assay and the one-stage clotting assay are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (+ 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, hepatitis B, hepatitis C, and HIV.
    - Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received. Any prior pharmacokinetics information obtained while the subject was receiving prophylactic or on-demand FVIII therapy prior to the study should also be collected. For subjects who have already enrolled in 270-201, this information should be collected at the next regularly scheduled study visit (or at least prior to the subject's completion of study participation).
- Complete Physical Examination
- Height and weight
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic substrate (plasma)
    - Baseline hFVIII activity level – one-stage clotting assay

  - hFVIII coagulation activity exploratory assay
  - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
  - hFVIII total antibody assay
  - hFVIII antigen (ELISA)
- Blood sample for AAV5 Assays
  - AAV5 antibody titer
  - AAV5 transduction inhibition assay
- Screen for hepatitis B, hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1)
- Von Willebrand Factor Antigen (VWF:Ag)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- Blood sample for AAV5 Assays

  - AAV5 antibody titer
  - AAV5 transduction inhibition assay
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1)
- PBMC collection for CTL baseline
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
  - AAV5 antibody titer
  - AAV5 transduction inhibition assay

- BMN 270 Infusion
- Vital Signs
    - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Once per week (Weeks 1 through 16)

The following procedures will be performed at one visit per week from Weeks 1 through 16:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- Samples for FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16

- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- PBMC collection

12.5.2 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only) – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Liver Tests (refer to Table 9.7.9.3.1) (Day 4 only) – central laboratory assessment
- Liver Tests (refer to Table 9.7.9.3.1) (Day 4 only) – local laboratory assessment
- FVIII activity level (Day 4 only) – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

12.5.3 Week 1 – Day 8

On Day 8, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Direct Thrombin test
- Haemo-QoL-A QoL assessment

12.5.4 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1) (not assessed at Week 14)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2)
    - Collection to occur until at least 3 consecutive negative results are obtained. Semen samples should continue to be collected and tested through Week 12, even if 3 consecutive negative results in that compartment have been recorded prior to that timepoint.

12.5.5 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedures will be performed:

- Urine Tests (refer to Table 9.7.9.2.1)
- VWF:Ag
- FVIII antibody titer
- Exploratory biomarker assessments

12.5.6 Week 16

At Week 16, the following procedures will be performed:

- Test for hepatitis B and hepatitis C reactivation (in subjects with a history of hepatitis B or hepatitis C infection prior to study entry)
    - Subjects who receive reactive corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

12.5.7 Weeks 8 and 16

At Weeks 8 and 16, the following procedures will be performed:

- AAV5 antibody titer

12.5.8 Weeks 2, 3, 4, and 16

At Weeks 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.5.9 Week 13

At Week 13, the following procedure will be performed:

- Direct Thrombin test

12.6 Safety Follow-Up – Weeks 17-36

After the Post-Infusion Follow-Up visits are complete, subjects will return to the study site for Safety Follow-Up visits from Weeks 17 through Week 36 (± 48 hours), when the following procedures will be completed:

12.6.1 Once per week (Weeks 17 through 36)

Once per week from Week 17 through Week 36, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

12.6.2 Once per week (Weeks 17 through 20)

Once per week from Week 17 through Week 20, the following procedures will be performed:

- PBMC collection

12.6.3 Every 2 weeks (Weeks 21 through 36)

Every 2 weeks (Weeks 22, 24, 26, 28, 30, 32, 34, and 36), the following procedures will be performed:

- PBMC collection

12.6.4 Every 4 Weeks

Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- VWF:Ag
- AAV5 antibody titer
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.5 Every 8 Weeks

Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed:

- FVIII antibody titer

12.6.6 Week 26

At Week 26, the following procedure will be performed:

- Direct Thrombin test

- Weight

12.6.7 Week 28

At Week 28, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Weeks 37-52

Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:

12.7.1 Once per visit

At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level

- FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

12.7.2 Every 4 Weeks

Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- AAV5 antibody titer
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
  - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.7.3 Every 8 Weeks

Every 8 weeks (Weeks 44 and 52), the following procedure will be performed:

- PBMC collection
- FVIII antibody titer

12.7.4 Week 38 and 52

At Week 38 and Week 52, the following procedure will be performed:

- Direct Thrombin test

12.7.5 Week 52

At Week 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment
- Weight

12.8 Safety Follow-Up – Years 2-5

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 and 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, follow an abbreviated visit schedule after Week 52 of the study by attending only the Q12W and End of Year visits during Years 2-5.

Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from semen must still provide samples for assessment every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until vector shedding has cleared (but do not have to perform other scheduled assessments at those study visits).

At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study.

During Years 2-5 of Safety Follow-up, the following procedures will be completed:

12.8.1 Year 2 – Every 4 Weeks (not required for treatment failure)

During Year 2, every 4 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Year 2 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks during Year 2 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).
    - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks during Year 2 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.2 Years 3-5 – Every 6 Weeks (not required for treatment failure)

During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be assessed more frequently when ALT values are >1.5x ULN or based upon discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be assessed more frequently when ALT values are >1.5x ULN or based upon discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Years 3-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52 and/or Year 2. Subjects who have not had 3 consecutive negative semen samples by the end of Year 2 should continue to have PCR testing of semen every 6 weeks during Years 3-5 until 3 consecutive samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).
    - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from semen, must still provide samples for assessment every 6 weeks during Years 3-5 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.3 Years 2-5 – Every 12 Weeks and End of Year Visits (required for all subjects)

During Years 2-5, subjects will be asked to return to the study site for visits at the following study weeks (±2 weeks):

- Year 2 – Week 64, Week 76, Week 88, Week 100, Week 104
- Year 3 – Week 116, Week 128, Week 140, Week 152, Week 156
- Year 4 – Week 168, Week 180, Week 192, Week 204, Week 208
- Year 5 – Week 220, Week 232, Week 244, Week 256, Week 260

For each of these years, the last study visit listed (Week 104, Week 156, Week 208, and Week 260) will serve as an End of Year visit.

At the every 12 week and End of Year visits, the following procedures will be performed:

- Brief physical examination (at every 12 week visits) or complete physical examination (at End of Year visits) (genitourinary examination may be deferred unless the subject has genitourinary-related complaints)
- Weight (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be assessed more frequently when ALT values are >1.5x ULN or based upon discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be assessed more frequently when ALT values are >1.5x ULN or based upon discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Vital Signs

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
    - ABO blood typing assessment should be performed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study)
- Urine Tests (refer to Table 9.7.9.2.1)
- FVIII antigen (ELISA)
- AAV5 antibody titer (at End of Year visits only)
- FVIII antibody titer (at End of Year visits only)
- PBMC collection
- VWF:Ag
- Exploratory biomarker assessments
- Haemo-QoL-A QOL assessment (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
- Liver ultrasound (at End of Year 5 visit only)
    - Additional liver ultrasounds may be performed at interim time points (ie, between the End of Year visits) at the discretion of the Investigator.
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Years 2-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).

12.9 Safety Follow-Up – Years 6-7

If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

During Years 6-7 of Safety Follow-up, the following procedures will be completed:

12.9.1 Years 6-7 – Every 13 Weeks

Every 13 weeks (±4 weeks) during Years 6-7, the site should contact the subject remotely to collect the following data:

- Adverse events

- New or changed concomitant medications
- Diary entries (FVIII usage and bleeding events)

12.9.2 Years 6-7 – Every 26 Weeks and End of Year Visits

During Years 6-7, the following assessments will be performed at a site visit every 26 weeks (±4 weeks) (Week 286 and Week 338) and at the End of Year Visits (±4 weeks) (Week 312 and Week 364):

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Complete Physical Examination (genitourinary examination may be deferred unless the subject has genitourinary-related complaints) (Week 312 and Week 364 only)
- Weight (Week 312 and Week 364 only)
- Vital signs (Week 312 and Week 364 only)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be assessed more frequently when ALT values are >1.5x ULN or based upon discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- FVIII antigen (ELISA)
- AAV5 antibody titer (Week 312 and Week 364 only)
- FVIII antibody titer (Week 312 and Week 364 only)
- Exploratory biomarker assessments (Week 312 and Week 364 only)
- Haemo-QoL-A QOL assessment

- Liver ultrasound (Week 312 and Week 364 only)
    - Additional liver ultrasounds may be performed at interim time points at the discretion of the Investigator.
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Years 6-7 is not required if at least 3 consecutive samples are below the limit of detection as of the end of Year 5.

12.10 Liver Biopsy Substudy - Years 1-7

For the optional liver biopsy substudy (refer to Table 9.1.7), the following procedures will be performed at the following times:

- Within 28 days before Biopsy Day
    - Informed consent
    - Liver ultrasound
        - Liver ultrasound does not need to be performed if an ultrasound result from the prior 3 months is already available
    - Brief physical examination
    - Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.9.2.1)
    - Liver Tests (refer to Table 9.7.9.3.1)
- Within 7 Days before Biopsy Day
    - FibroScan
    - Local laboratory FVIII activity level assessment
    - Pre-biopsy consultation with investigator (treating hematologist). This may need to be repeated/extended if the subject's FVIII activity levels need adjustment before the biopsy, at the discretion of the investigator (treating hematologist).
- 1 Day before Biopsy Day
    - Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.9.2.1)
    - Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - Brief physical examination

- Local laboratory FVIII activity level assessment
  - Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".

- Biopsy Day
  - Brief physical examination
  - Local laboratory FVIII activity level assessment (performed pre-biopsy)
    - Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".
  - Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment (pre-biopsy)
  - Liver Biopsy (refer to Laboratory Manual for guidelines for biopsy procedure and handling of biopsy samples)
    - If only a small amount of tissue is obtained at the time of the biopsy, the subject may be asked to consent for a second pass.

Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion.

Participation in the substudy may include liver biopsies at one or more time points.

12.11 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 364 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag

- Liver ultrasound
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if at least 3 consecutive samples are below the limit of detection prior to the ETV.
- Exploratory biomarker assessment
- Haemo-QoL-A QOL assessment

12.12 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 364). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at $\geq$ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay as measured by the central laboratory at 16 weeks following study treatment. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Liver Biopsy Substudy Analysis

A separate report presenting and discussing analyses of the exploratory objectives for the optional liver biopsy substudy will be prepared.

14.4 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.5 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by a one-stage clotting assay and/or chromogenic substrate assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.6 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.7 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.8 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.9 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IEC must be sought, and the Investigator should inform BioMarin and the full IEC within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IEC must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IEC prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IEC, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COSTS, COMPENSATION, AND SUBJECT INJURY

BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after IEC approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries. If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study subject's primary disease or any concurrent disease and that are unrelated to this study.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked.

Upon completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas AND to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees. When in person site monitoring or source data verification cannot be conducted, remote site monitoring and/or source data verification will be conducted where allowed by country and local health authorities and IECs.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572 and/or principles of ICH E6 R2 GCP, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and/or ICH E6 R2 Sections 2.9 and 4.8 are met. As well, he or she will ensure that IEC review and approval in 21 CFR Part 56 and/or ICH E6 R2 Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH E6 R2 Section 4.11.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH E6 R2 Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH E6 R2 Section 4.9.7.
- The IEC complies with the requirements of 21 CFR Part 56, ICH E6 R2 Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IEC. Additionally, he or she will not make any changes in the research without IEC approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201 Amendment 10

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including ICH E6 R2, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name _____

Accepted for the Sponsor:

DocuSigned by:
PI
Signer Name: PI
Signing Reason: I approve this document
Signing Time PI
934DEBF504AB4E6A83363FE5BE4B8D5F

Medical Monitor Signature Date

Printed name: PI MD, PhD, PI , Clinical Sciences

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See page 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Revision	Rationale
2/Synopsis (Study Design and Plan)	In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as ~~either~~ failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 ~~or~~and 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator. … **Liver Biopsy Substudy Design** Participation in the sub-study may include liver biopsies at one or more time points.	1, 5, 9
5.1/~~Institutional Review Board or~~ Independent Ethics Committee	Prior to initiating the study, the Investigator will obtain written confirmation that the ~~institutional review board (IRB) or~~ independent ethics committee (IEC) ~~[for Canadian protocols, Research Ethics Board (REB)]~~ is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the ~~IRB~~/IEC/~~REB~~ will be provided to BioMarin or its designee. The Investigator will provide the ~~IRB~~/IEC/~~REB~~ with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the ~~IRB~~/IEC/~~REB~~ confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the ~~IRB~~/IEC/~~REB~~ and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.	9
5.2/Ethical Conduct of Study	Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an ~~IRB~~/IEC/~~REB~~ and will be conducted by scientifically and medically qualified persons.	9

Section No./Title	Revision	Rationale
5.3/Subject Information and Informed Consent	A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the ~~IRB/~~IEC/~~REB~~ approval. BioMarin and the ~~IRB/~~IEC/~~REB~~ must approve the documents before they are implemented.	9
7/Introduction	Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (~~Nathwani, 1992~~Iorio 2019). … Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava~~, 2013~~ 2020); thus the therapeutic goal for gene therapy is a modest increase in hFVIII.	9
7.2/Previous and Ongoing Clinical Studies	Ongoing clinical studies for BMN 270 include: • 270-203, a phase 2 study in patients with severe HA who have anti-AAV5 antibody titers • 270-205, a phase 1/2 study in patients with severe HA who have active or prior FVIII inhibitors • 270-301, the pivotal phase 3 study in patients with severe HA who receive BMN 270 at the 6E13 vg/kg dose level • 270-302, a phase 3 study in patients with severe HA who receive BMN 270 at the 4E13 vg/kg dose level • 270-303, a phase 3 study in patients with severe HA who received BMN 270 at the 6E13 vg/kg dose level along with prophylactic corticosteroids A comprehensive review of safety, efficacy, and immunogenicity results as of the latest data cut is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.	9
7.3/Study Rationale	Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (~~Mannucci, 2001~~Iorio 2019).	9
7.4/Summary of Risks and Benefits	Transient, asymptomatic ALT elevation (grade 1 to 3 in severity) was observed in most subjects administered BMN 270 shortly after dosing, with no symptoms or sequelae suggestive of clinically significant hepatocyte injury or liver dysfunction. ~~In almost all subjects, ALT elevations decreased quickly following corticosteroid treatment. There were differences in~~Across the ~~use~~6E13 vg/kg cohort of ~~corticosteroids across studies. Subjects in~~ 270-201~~, who received corticosteroids an average of 8 weeks earlier following BMN 270 infusion than the mITT population~~ and 270-301, subjects enrolled in 270-~~301, were more likely to avoid a significant decline in FVIII activity concurrently with an~~201 developed ALT elevation~~, and saw a more robust recovery of FVIII activity upon~~ about 5.5 weeks later than subjects in 270-301, generally once the first ~~use~~course of	8, 9

Section No./Title	Revision	Rationale
	corticosteroids~~,~~ was being tapered, and experienced lower peak elevations in ALT (75.7 U/L) than ~~did the~~ subjects in 270-301 (112.5 U/L). The difference in the ~~mITT population in 270-301. Despite the clinical response to steroids, no associations~~ALT profile seen between ~~safety parameters (transient ALT rises), or efficacy as measured~~the 6E13 vg/kg subjects in 270-201 and the subjects in 270-301 could be attributed to the difference in the protocol-specified corticosteroid regimens in place in those studies, including the early use of corticosteroids (ie, by ~~FVIII activity levels were found~~Week 3 post-BMN 270 infusion). While the majority of ALT elevations responded rapidly to corticosteroids, given current interest in the field of AAV gene therapy for the use of non-steroidal approaches to ~~be temporally associated with anti-AAV5 antibody~~managing or preventing ALT elevations, alternate non-steroidal systemic immunosuppressive agents have also been used to manage hepatic reactions where corticosteroids have proven to be ineffective or ~~cellular immune responses.~~where high doses/and or prolonged exposure to corticosteroids have led to unwanted side effects. Overall, the literature and clinical experience with BMN 270 suggests that transient elevations in liver enzymes are expected following AAV-based gene therapy for the treatment for hemophilia A or B without any long-term concerns of hepatic injury (Manno 2006; Nathwani 2011; George 2016; Miesbach 2016; Pasi 2020).	
9.1/Overall Study Design and Plan	In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as ~~either~~ failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 ~~or~~and 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.	5
Table 9.1.5/Schedule of Events Years 2-7	Table 9.1.5 has been updated consistent with changes made in the footnotes and elsewhere in the protocol.	1, 6, 9
Table 9.1.5 footnotes	ETV: Early Termination Visit [a] Complete physical examination should be performed at the End of Year visits~~;~~ (genitourinary examination may be deferred unless the subject has genitourinary-related complaints); brief physical examination may be performed at other study visits. Weight should be recorded at the second Q12W visit each year during Years 2-5, and at every End of Year visit during Years 2-7. [b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be ~~monitored~~assessed more ~~or less~~ frequently ~~(and in particular~~ when ALT values are ≥ 1.5x ULN~~)~~ or based ~~on~~upon discussion between the Medical Monitor and the Investigator ~~and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients~~. Subjects with ALT ≥ 1.5x ULN during the study may	1, 6, 9

Section No./Title	Revision	Rationale
	undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment~~; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or~~;; or (2) after a discussion between the Medical Monitor and the Investigator based on a review of subject data. ~~If FVIII levels and/or~~If ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. During Years 2-5 of the Post-Infusion Follow-Up period, urine tests and blood, chemistry, and coagulation tests should be performed at the second Q12W visit each year and at every End of Year visit. During Years 6-7, these tests should be performed at the Q26W and End of Year visits. [d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, and hFVIII coagulation activity exploratory assay ~~and hFVIII antigen (ELISA)~~. [e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), and/or every 26 weeks (during Years 6-7) until 3 consecutive samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor). [h] Subjects who meet the definition of treatment failure to BMN 270 therapy after Week 52 may omit the Q4W and Q6W visits during Years 2-5, and must attend only the Q12W and End of Year visits. Such subjects following the abbreviated schedule who have not yet cleared vector shedding in ~~a fluid~~semen must still provide samples Q4W (during Year 2), Q6W (during Years 3-5), or Q26W (during Years 6-7), until vector shedding has been cleared (but do not need to do other scheduled assessments at those visit dates), either by reporting to the site to provide samples or by providing those samples to an MN professional. [k] Additional liver ultrasounds may be performed at interim timepoints (ie, between the End of Year visits) at the discretion of the Investigator.	
Table 9.1.6/~~Therapeutic~~ Reactive Corticosteroids	Table 9.1.6 has been update consistent with changes made elsewhere in the protocol and in the footnotes.	4, 9
Table 9.1.6 footnotes	[a] ~~Therapeutic~~Reactive corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor. [b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may	4, 9

Section No./Title	Revision	Rationale
	take into consideration timing of the ALT elevation (after Week 52), as well as possible confounders for the ALT elevation and adverse events related to corticosteroid dosing ~~impact on FVIII expression.~~. Guidance for tapering oral corticosteroids can be found in Section 9.4.8.2.	
9.4.8/Prior and Concomitant Medications	Administration of SARS-CoV-2 vaccine after BMN 270 infusion may occur after consultation between Investigator and Medical Monitor. Investigators should use clinical judgment, taking into consideration local factors, individual risk factors, and the benefit/risk related to timing of vaccine administration.	3
9.4.8.1/Concomitant Hemophilia Treatments	Subjects on "~~on demand~~episodic" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "~~on demand~~episodic" schedule.	9
9.4.8.2.1/~~Therapeutic~~ Reactive Corticosteroids	In general, ~~therapeutic~~reactive corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator. Corticosteroids may be delayed, upon discussion with the Medical Monitor, if elevations in ALT are clearly not related to BMN 270 (eg, elevated ALT with concurrent increase in CPK due to intensive exercise) and in particular for elevations occurring more than 52 weeks after BMN 270 dosing. … Following initiation or completion of ~~therapeutic~~reactive corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression. Unless otherwise indicated, treatment with ~~prednisolone will~~reactive corticosteroids should be initiated at a dose of 60 mg per day and then gradually tapered if the ALT level remains stable or declines after 2 weeks (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.6). Should a scenario arise in which differences from the minimum recommended dose and/or duration of reactive corticosteroids may be clinically indicated, a discussion should take place between the Investigator and Medical Monitor regarding corticosteroid dose adjustments. Management of ALT elevations with reactive corticosteroids, including tapering of doses and managing worsening and/or recurrent ALT elevations, should be guided by the following (Table 9.4.8.2.1.1):	4, 9
Table 9.4.8.2.1.1/ Management of ALT Elevations with Corticosteroids	Table 9.4.8.2.1.1 and its footnotes have been added.	4

Section No./Title	Revision	Rationale
9.4.8.2.1/~~Therapeutic~~ Reactive Corticosteroids (cont.)	When ruling out alternative viral or autoimmune hepatitis as part of the elevated ALT workup, the following tests should be performed (Table 9.4.8.2.1.2):	4
Table 9.4.8.2.1.2/Viral and Autoimmune Hepatitis Testing	Table 9.4.8.2.1.2 has been added	4
9.4.8.2.1/~~Therapeutic~~ Reactive Corticosteroids (cont.)	After discontinuation of reactive corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. The use of COX-2 inhibitors, while not contraindicated during corticosteroid treatment, should be limited, if possible. Practical management to prevent complications related to oral corticosteroid therapy may be undertaken at the discretion of the Investigator (eg, evaluation of glucose intolerance, hyperlipidemia etc.). For the subjects with a past medical history of hepatitis B or hepatitis C prior to study entry, Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol. Subjects on corticosteroids should receive appropriate counselling and support regarding side effects from the Investigator or the treating institution (eg, listings of side effects and when to notify carers, wallet card for emergencies if on steroids, etc.). Additional management, including the co-prescription of additional medications to prevent complications related to corticosteroid therapy, may be undertaken at the discretion of the investigator, including, but not limited to, prophylaxis against the occurrence of gastric ulcers, osteoporosis, and infections.	4, 9
9.7.2/Primary Efficacy Variables	FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5 but will be used in conjunction with local lab LT assessments to monitor subject safety and need for initiation of ~~therapeutic~~reactive corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as ~~either~~ failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 ~~or~~and inability to maintain independence from prophylactic FVIII replacement therapy due to	4, 5

Section No./Title	Revision	Rationale
	joint bleeding episodes, in the judgment of the Investigator) may, at the Investigator's discretion and after discussion with the Medical Monitor, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.	
9.7.8/Liver Biopsy Substudy	Participation in the substudy may include liver biopsies at one or more time points.	1, 9
9.7.9.3/Malignancies	Liver ultrasounds will be performed annually at each End of Year visit starting at Year 5 (Week 260) through the end of the study to screen for HCC. Additional liver ultrasounds may be performed between the End of Year visits at the discretion of the Investigator. Any development of a malignancy (except non-melanoma skin cancers) during the course of the study will be considered an EOSI (refer to Section 10.2.1) and is subject to expedited reporting. In addition, it is recommended that genomic analyses be performed on any malignancy (except non-melanoma skin cancers) diagnosed during the course of the study. The study site will coordinate sending samples from the malignancy for genomic analyses, if available.	1
9.7.8.6/Vital Signs and Physical Exams	A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and at the End of Years visits thereafter. A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems. The genitourinary examination may be deferred for visits after Year 1 unless the subject has genitourinary-related complaints.	9
9.7.9.7/Vector Shedding	Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), and every 26 weeks (during Years 6-7) until 3 consecutive semen samples below the limit of detection of the test are documented (or upon consultation between the Investigator and Medical Monitor). Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule (refer to Section 12.5.3 but who have not cleared vector shedding from ~~all fluids~~semen must still provide samples for assessment until vector shedding has cleared: every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), or every 26 weeks (during Years 6-7), either by reporting to the site to provide samples or by providing those samples to an MN professional.	9
10.2.1/EOSI	The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:	2

Section No./Title	Revision	Rationale
	• Any new diagnosis of malignancy (except non-melanoma skin cancer)	
10.8/Urgent Safety Measures	The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours of becoming aware of the event.	9
10.9/Contact Information	The medical monitor contact information has been updated	7
12.8/Safety Follow-Up Years 2-5	Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as ~~either~~ failure to achieve FVIII activity ≥ 5 IU/dL by Week 52 ~~or~~and 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, follow an abbreviated visit schedule after Week 52 of the study by attending only the Q12W and End of Year visits during Years 2-5. Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from ~~a fluid~~semen must still provide samples for assessment every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until vector shedding has cleared (but do not have to perform other scheduled assessments at those study visits).	5, 9
12.8.2/Years 3-5 Every 6 Weeks	During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed: • Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment o LTs may be ~~monitored~~assessed more ~~or less~~ frequently ~~(and in particular~~ when ALT values are >1.5x ULN~~)~~ or based ~~on~~upon discussion between the Medical Monitor and the Investigator and review of subject data. • Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment o LTs may be ~~monitored~~assessed more ~~or less~~ frequently ~~(and in particular~~ when ALT values are >1.5x ULN~~)~~ or based ~~on~~upon discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII Assays – central laboratory assessment ~~o FVIII antigen (ELISA)~~	4, 6, 9

Section No./Title	Revision	Rationale
	• PCR of vector DNA in blood, saliva, urine, semen, and stools (if required) o Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from ~~fluid~~semen, must still provide samples ~~of that fluid~~ for assessment every 6 weeks during Years 3-5 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).	
12.8.3/Years 3-5 Every 12 Weeks and End of Year Visits	At the every 12 week and End of Year visits, the following procedures will be performed: • Brief physical examination (at every 12 week visits) or complete physical examination (at End of Year visits) (genitourinary examination may be deferred unless the subject has genitourinary-related complaints) • Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment o LTs may be ~~monitored~~assessed more ~~or less~~ frequently ~~(and in particular~~ when ALT values are >1.5x ULN~~)~~ or based ~~on~~upon discussion between the Medical Monitor and the Investigator and review of subject data. • Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment o LTs may be ~~monitored~~assessed more ~~or less~~ frequently ~~(and in particular~~ when ALT values are >1.5x ULN~~)~~ or based ~~on~~upon discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII Assays – central laboratory assessment ~~o FVIII antigen (ELISA)~~ • FVIII antigen (ELISA) • AAV5 antibody titer (at End of Year visits only) • FVIII antibody titer (at End of Year visits only) • Liver ultrasound (at End of Year 5 visit only) o Additional liver ultrasounds may be performed at interim time points (ie, between the End of Year visits) at the discretion of the Investigator.	1, 4, 6, 9
12.9.2/Years 6-7 Every 26 Weeks and End of Year	During Years 6-7, the following assessments will be performed at a site visit every 26 weeks (± 4 weeks) (Week 286 and Week 338) and at the End of Year Visits (± 4 weeks) (Week 312 and Week 364):	1, 4, 6, 9

Section No./Title	Revision	Rationale
	• Complete Physical Examination (genitourinary examination may be deferred unless the subject has genitourinary-related complaints) (Week 312 and Week 364 only) • Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment o LTs may be ~~monitored~~assessed more ~~or less~~ frequently ~~(and in particular~~ when ALT values are >1.5x ULN~~)~~ or based ~~on~~upon discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII Assays – central laboratory assessment ~~o FVIII antigen (ELISA)~~ • FVIII antigen (ELISA) • Liver ultrasound (Week 312 and Week 364 only) o Additional liver ultrasounds may be performed at interim time points at the discretion of the Investigator.	
12.10/Liver Biopsy Substudy	Participation in the substudy may include liver biopsies at one or more time points.	1, 9
12.11/ETV	At the Early Termination visit, as many of the following assessments as possible should be done: • FVIII Assays – central laboratory assessment ~~o FVIII antigen (ELISA)~~ • FVIII antigen (ELISA) • Liver ultrasound	1, 6
14.9/Changes in Study Conduct	Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the ~~IRB/~~IEC/~~REB~~ must be sought, and the Investigator should inform BioMarin and the full ~~IRB~~/IEC/~~REB~~ within 2 working days after the emergency occurs. With the exception of minor administrative or typographical changes, the ~~IRB~~/IEC/~~REB~~ must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the ~~IRB~~/IEC/~~REB~~ prior to their implementation.	9

Section No./Title	Revision	Rationale
	When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the ~~IRB/~~IEC/~~REB~~, and all active subjects must again provide informed consent.	
16/Costs, Compensation, and Injury	BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after ~~IRB/~~IEC/~~REB~~ approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.	9
18/Study Monitoring	When in person site monitoring or source data verification cannot be conducted, remote site monitoring and/or source data verification will be conducted where allowed by country and local health authorities and IECs.	9
21/References	George LA, Sullivan S, Teitel J, Cuker A et al. Preliminary results of a phase 1/2 trial of SPK-9001, a hyperactive FIX variant delivered by a novel capsid, demonstrate consistent factor IX activity levels at the lowest dose cohort. Haemophilia. 2016;22(S4):151-152. ~~Heller CG & Clermont Y. Kinetics of the germinal epithelium in man. Recent Prog Horm Res. 1964;20:545-571.~~ ~~Heller CG, Heller GV, Rowley MJ. Human spermatogenesis: an estimate of the duration of each cell association and each cell type. Excerpta Med Int Congr Ser. 1969;184:1012-1018.~~ Iorio A, Stonebraker JS, Chambost H, et al, for the Data and Demographics Committee of the World Federation of Hemophilia. Establishing the Prevalence and Prevalence at Birth of Hemophilia in Males: A Meta-analytic Approach Using National Registries. Ann Intern Med. 2019;171(8):540-546. ~~Monahan, P, Walsh CE, Powell JS, Konkle BA et al. Update on a phase 1/2 open-label trial of BAX335, an adeno-associated virus 8 (AAV8) vector-based gene therapy program for hemophilia B. J Thromb Haemostasis 13[Suppl 2], 1-997. 2015.~~ ~~Nathwani, AC, Tuddenham, EG. Epidemiology of coagulation disorders. Baillieres Clin Haematol 5[2], 383-439. 1992.~~ National Hemophilia Foundation. MASAC recommendation for liver biopsies in gene therapy trials for hemophilia. Available at https://hemophilia.org/sites/default/files/ document/files/256.pdf. 2019. MASAC Recommendation #256. Last accessed ~~5 June 2020~~20 July 2021. Pasi KJ, Rangarajan S, ~~Walsh L~~Mitchell N, Lester W et al. Multiyear Follow-up of AAV5~~-Factor-~~hFVIII-SQ Gene ~~Transfer in Severe~~Therapy for Hemophilia A. ~~NEJM. 2017;377(26):2519-2530~~N Engl J Med. 2020;382(1):29-40. Srivastava~~,~~ A, ~~Brewer, AK, Mauser-Bunschoten, EP, Key, NS~~Santagostino E, Dougall A, Kitchen S et. al. WFH guidelines for the management of hemophilia~~.~~, 3rd edition. Haemophilia ~~19~~[. 2020;26(Suppl 6):1]~~, e1-47. 2013~~-158.	9

Section No./Title	Revision	Rationale
	~~Watkins, PB, Kaplowitz, N, Slattery, JT, Colonese, CR et al. Aminotransferase Elevations in Healthy Adults Receiving 4 Grams of Acetaminophen Daily: A Randomized Controlled Trial. JAMA 296[1], 87-93. 2006.~~ ~~White, GC, Rosendaal, F, Aledort, FM, Lusher, JM et al. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International society on Thrombosis and Haemostasis. Thromb Haemost. 85[3], 560. 2001.~~	
22.1/Conduct of Study	In accordance with FDA Form 1572 and/or principles of ICH E6 R2 GCP, the Investigator will ensure that: • He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and/or ICH E6 R2 Sections 2.9 and 4.8 are met. As well, he or she will ensure that ~~IRB/~~ IEC review and approval in 21 CFR Part 56 and/or ICH E6 R2 Section 2.6 are met. • The ~~IRB/~~IEC/~~REB~~ complies with the requirements of 21 CFR Part 56, ICH E6 R2 Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the ~~IRB/~~IEC/~~REB~~. Additionally, he or she will not make any changes in the research without ~~IRB/~~IEC/~~REB~~ approval, except where necessary to eliminate apparent immediate hazards to human patients.	9
23/Signature Page	Printed name: ~~Nina Mitchell, MA MB BChir MSc,~~Tara Robinson, MD, PhD, Associate Medical Director, Clinical ~~Science~~Sciences	7

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI , MA MB BChir MSc BioMarin (UK) Ltd. 10 Bloomsbury Way London, WC1A 2SL
Duration of Subject Participation:	Approximately 368 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015
Date of Amendment 4:	02 September 2016
Date of Amendment 5:	14 February 2017
Date of Amendment 6:	21 December 2017
Date of Amendment 7:	10 October 2018
Date of Amendment 8:	31 January 2019
Date of Amendment 9:	19 June 2020

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 9

Date: 19 June 2020

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 9 to the 270-201 protocol is provided below.

1. The duration of the study has been extended from 5 years to 7 years post-infusion.

Rationale: Additional monitored follow-up will permit long-term analysis for key study objectives and increased long-term safety monitoring. The schedule of assessments for the additional 2 years has been reduced compared with Years 2-5, in order to minimize subject burden (only 2 clinic visits per year will be required).

2. Language has been added to permit the use of mobile nursing (MN) services, provided that the subject consents and that the site can implement the use of such services.

Rationale: Allowing for the use of MN services at study timepoints with a limited number of assessments will help alleviate subject travel burden and will allow for the collection of key data points where COVID-related travel and site visit restrictions exist. At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office.

3. Lamivudine has been removed as a prohibited medication.

Rationale: 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 and, as such, should no longer be listed as a prohibited medication.

4. The occurrence of events of Hy's law has been added as an event of special interest (EOSI) for purposes of expedited safety reporting.

Rationale: Events potentially meeting the criteria for Hy's law involve combined assessment of elevations in aminotransferases and total bilirubin levels, while the current list of EOSI focuses on elevations in aminotransferases. To date, no events meeting the criteria for Hy's law have been reported in any BMN 270 study. While monitoring for events of Hy's law has been ongoing as part of routine pharmacovigilance in all BMN 270 studies, this change

ensures that the occurrence of any events in the future will be reported in an expedited manner.

5. The development of anti-FVIII inhibitory antibodies (inhibitors) has been added as an EOSI for purposes of expedited safety reporting.

Rationale: To date, no subjects receiving BMN 270 have developed FVIII inhibitors in any BMN 270 study. While monitoring for the development of inhibitors has been ongoing as part of routine pharmacovigilance in all BMN 270 studies, this change ensures that the occurrence of any events in the future will be reported in an expedited manner.

6. Language has been added concerning the use of liver biopsy sample information from samples collected outside of the liver biopsy substudy.

Rationale: Where a biopsy has been taken for safety-related reasons (rather than as part of the liver biopsy substudy) or was available from a past procedure, the Sponsor may want to see that biopsy information to help evaluate the impact of BMN 270 on the liver. The Sponsor may request that slides from a liver biopsy not performed as part of the substudy be made available for additional histopathological review.

7. Vector shedding and contraception use language has been updated to change the determination of a “clear” result from negative to below the limit of detection.

Rationale: The change in language better reflects regulatory guidance documents and aligns 270-201 with other clinical studies in the BMN 270 program.

8. Provision has been included to allow an investigator to document verbal confirmation by a subject that has signed and dated the written informed consent when the investigator cannot obtain a copy of the signed informed consent prior to initiating study procedures.

Rationale: Travel and site restrictions related to COVID-19 may make it impossible for a subject to go to the site to provide written informed consent. In such a case, the Investigator should have the subject verbally confirm during an informed consent interview that the subject has signed and dated the informed consent form (ICF). The Investigator should document this verbal confirmation on the Investigator's copy of the ICF and, when it is possible to do so, receive the informed consent signed by the subject and archive the original(s) in the record file of the subject. Since the study has already fully recruited, this process will only be used in currently enrolled subjects providing consent to updated versions of the ICF, and there is no intention that this will be used for the initial consenting process.

9. Guidance concerning how to determine whether a subject has been lost to follow-up has been added.
10. The vector genome schematic has been updated.
11. The costs and compensation language has been updated to clarify which study-related costs will be covered by the Sponsor.

12. The summary of risks and benefits (Section 7) has been updated.
13. Minor administrative changes have been made for consistency and clarity.

Specific changes included in this amendment, including the Synopsis, since Amendment 8 (approved 31 January 2019) are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients.

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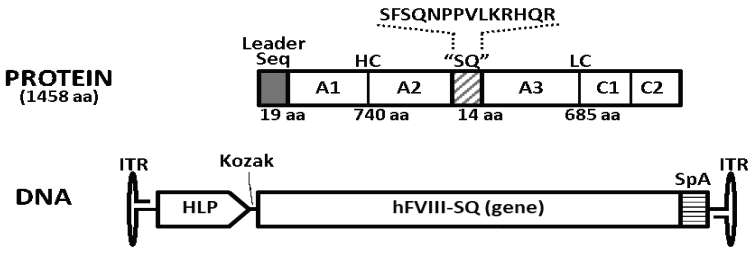
There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 1).

Figure 1. hFVIII-SQ Vector Genome and Encoded Protein



Legend –Note that schematic is not to scale; aa = amino acids; ITR = inverted terminal repeat; HLP = human liver promoter; Kozak = Kozak concensus sequence (GCCACC); SpA = Synthetic poly(A) signal

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BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

Liver Biopsy Substudy Rationale

The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms, and it is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans.

Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown.

The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to circularized genomes, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.

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OBJECTIVES:

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

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 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)

STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 7 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

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Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)

Cohort 2: 2E13 vg per kilogram, iv

Cohort 3: 6E13 vg per kilogram, iv

Cohort 4: 4E13 vg per kilogram, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in non-human primates.

Cohorts 1-3

The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the one-stage clotting and chromogenic substrate assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥ 5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator. Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 7 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

Liver Biopsy Substudy Design

All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy).

Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL).

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NUMBER OF SUBJECTS PLANNED:

Up to 15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive semen samples below the limit of detection of the test.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4

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5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria:

1. Currently enrolled in 270-201
2. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy
3. Able to sign informed consent and comply with requirements of the substudy
4. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy:

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1. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to):
   - Significant thrombocytopenia (platelet count < 100 x 10^9/L)
   - Evidence of significant ascites
   - Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous infusion.

CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver tests (LTs, including ALT, AST, GGT, LDH, total bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52) during Year 1. LTs will be monitored every 4 weeks during Year 2, every 6 weeks for Years 3-5, and every 26 weeks for Years 6-7 post-dose in the safety extension; the frequency and duration of LT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

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Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage clotting and/or chromogenic substrate assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

Liver Biopsy Substudy:

The following exploratory assessments will be performed as part of the optional liver biopsy substudy:

- Morphologic/pathogenic changes after FVIII gene transduction or any change that may be associated with sustained ALT rise
- Determine quantities of liver FVIII-SQ DNA/RNA
- Determine forms of vector DNA in liver at the time of biopsy
- Determine percentage of hepatocytes expressing FVIII protein
- Determine percentage of hepatocytes staining positive for vector DNA
- Determine hepatic liver transcriptome at single nuclei level if sufficient material is obtained
- Identify the forms of vector DNA in the liver
- Examination of potential stress inducing cellular pathways
- Other exploratory assessment to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint,

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defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay at 16 weeks following study treatment. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates. Analysis of neutralizing antibody response and other immunological parameters as well as vector shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.		

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
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
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee
IND	Investigational New Drug (application)

INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LT	liver test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen

Definition of Terms:

Investigational Product (IP):

“A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use” (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms “IP” and “study drug” may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written informed consent before any study-related tests or evaluations are performed, as well as when updates to the ICF are made. Where it is not feasible for the Investigator to receive the signed, written ICF from the subject prior to beginning new or changed study-related procedures contained in such an ICF update (for example, due to COVID-19-related restrictions), the Investigator will ask the subject to verbally confirm during an informed consent interview that the subject has signed and dated the ICF. The Investigator should document this verbal confirmation on the Investigator's copy of the ICF and, when it is possible to do so, receive the informed consent signed by the subject and archive the original(s) in the record file of the subject.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet and all ICFs translated to a language other

than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver tests (LTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman, 2013). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay, 2012).

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2013); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single

dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270

transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani, 2006). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003) and an AAV8 vector (Nathwani, 2011), (Nathwani, 2014). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola, 2014). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Mannucci, 2001). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading

to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010), (Mahlangu, 2014). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

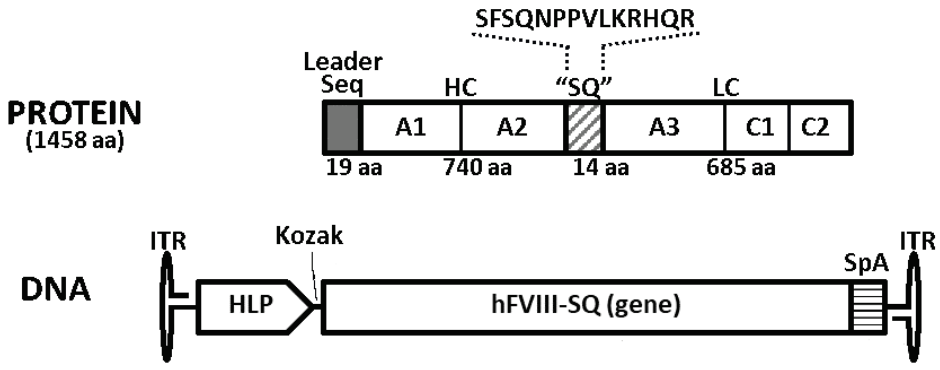
Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci, 2001).

They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005) for serotypes 2, 5 and 8 among others. Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, 2014). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011), (Bainbridge, 2008), (Maguire, 2009); (Simonelli, 2010).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: hFVIII-SQ Vector Genome and Encoded Protein



Legend –Note that schematic is not to scale; aa = amino acids; ITR = inverted terminal repeat; HLP = human liver promoter; Kozak = Kozak concensus sequence (GCCACC); SpA = Synthetic poly(A) signal

BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.3.1 Liver Biopsy Substudy Rationale

The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean

hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms. It is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans.

Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or the variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown. Finally, a call to incorporate liver biopsy sub-studies into gene therapy trials for hemophilia has been issued by medical and scientific leaders in the field to help illuminate these and other questions (National Hemophilia Foundation, 2019).

The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to genome circularization, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.

7.4 Summary of Overall Risks and Benefits

BMN 270 has an acceptable safety and tolerability profile that supports a positive benefit-risk assessment. Single infusions have been generally well tolerated by treated subjects across all investigated doses. All subjects have successfully completed their full-dose infusion of BMN 270, with no infusions requiring permanent termination prior to completion due to AEs. No deaths have been reported in any of the BMN 270 studies, and no participants discontinued from studies as a result of an AE. Frequency of adverse events decreased over time with no delayed adverse drug reactions.

Infusion reactions associated with BMN 270 administration included symptoms such as maculopapular rash, urticaria, nausea, diarrhea, watery eyes, rigors, chills, myalgia, fever, tachycardia and hypotension emerging within 24 hours of receiving BMN 270. All of these events subsided without clinical sequela within 48 hours following medical management

Infusion-related reactions were effectively mitigated by managing infusion rate and medications.

Transient, asymptomatic ALT elevation (grade 1 to 3 in severity) was observed in most subjects administered BMN 270 shortly after dosing, with no symptoms or sequelae suggestive of clinically significant hepatocyte injury or liver dysfunction. In almost all subjects, ALT elevations decreased quickly following corticosteroid treatment. There were differences in the use of corticosteroids across studies. Subjects in 270-201, who received corticosteroids an average of 8 weeks earlier following BMN 270 infusion than the mITT population in 270-301, were more likely to avoid a significant decline in FVIII activity concurrently with an ALT elevation, and saw a more robust recovery of FVIII activity upon the first use of corticosteroids, than did the subjects in the mITT population in 270-301. Despite the clinical response to steroids, no associations between safety parameters (transient ALT rises), or efficacy as measured by FVIII activity levels were found to be temporally associated with anti-AAV5 antibody or cellular immune responses.

At the highest dose tested in 270-201 (6E13 vg/kg), the majority of subjects achieved FVIII levels above 50 IU/dL at 52 weeks post-infusion. Subjects in that cohort also reported markedly decreased bleeding compared with pre-study rates and the ability to discontinue prophylactic FVIII infusions. Subjects at all dose levels continue to be followed.

In 270-301, an interim analysis has shown increased FVIII activity in the majority of subjects to mild HA or normal levels at 26 weeks post-infusion, also with markedly decreased bleeding compared with pre-study rates and the ability to discontinue prophylactic FVIII infusions. All subjects who will be included in the final analysis have been dosed with 6E13 vg/kg and continue to be followed.

The current data available has shown an established positive benefit-risk profile for BMN 270 at the 6E13 vg/kg dosing level. Given the monitoring measures in place in the clinical protocol(s) to minimize the risk to subjects participating in the existing studies, the identified risks are justified by the anticipated benefits that may be afforded to subjects. Each subject in 270-301 will have a comprehensive surveillance plan that monitors LTs during the study, and elevations in LTs will be addressed according to the guidelines set forth in the protocol. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

For additional information on safety findings in 270-201, refer to current version of the Investigator's Brochure.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose

6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

7.4.1 Optional Liver Biopsy Substudy Risks and Benefits

Liver biopsy is considered a safe procedure, with serious complications occurring less than once in every 10,000 procedures (Grant, 2004). Although the theoretical risks of significant complications are extremely small, the main complications would include bleeding and bile leakage. Another theoretical complication is infection at the needle insertion site; the sterile technique used makes this risk extremely small.

The most common problems include mild pain and a minor decrease in blood pressure. More serious complications, such as bleeding, infection, and injury to nearby organs, are very rare, but the subject will be monitored appropriately to ensure correct management should any of these occur. Any complications related to the liver biopsy should be reported as adverse events, as outlined in Section 10. The liver biopsy is a standard investigation, and will be explained more fully by the experienced clinician performing the biopsy.

Each subject who participates in this optional substudy will have a comprehensive pre-/post-biopsy surveillance plan according to the standard procedures at the institution. Safety will be assessed by adverse event reporting and clinical laboratory assessments. Per the Investigator's discretion and/or according to local guidelines, the subject may be kept in overnight following the liver biopsy for additional safety monitoring; such an overnight stay would not be considered a hospitalization for serious adverse event (SAE) reporting purposes (refer to Section 10.4.1.7).

There is no direct benefit from participating in this study other than contributing to understanding the mechanism of action of BMN 270. Consenting into this specific substudy is optional and will not have any effect on the subject’s continued participation in 270-201.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

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 rAAV genomic DNA (eg, concatemers) are present at the time of biopsy
- To determine the transduction pattern in humans (ie, peri-portal hepatocytes, central vein hepatocytes)

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 7 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

- Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
- Cohort 2: 2E13 vg per kilogram, iv
- Cohort 3: 6E13 vg per kilogram, iv
- Cohort 4: 4E13 vg per kilogram, iv

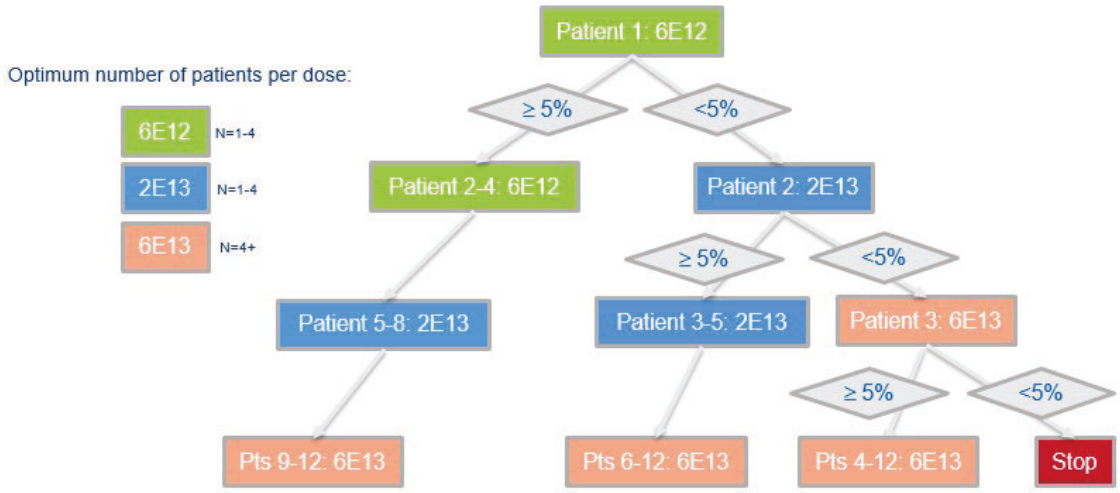
The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in mice.

Cohorts 1-3

The first three cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches ≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme for Cohorts 1 to 3



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the Data Review Board (DRB). The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 7 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected PBMC. Additionally, if any of the events listed in Section 9.3.4.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the DRB and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3, Table 9.1.4, and Table 9.1.5 for Safety Follow-up.

Table 9.1.6, dealing with therapeutic corticosteroid use in the event of elevated LTs, is discussed in Section 9.4.8.2.

Liver Biopsy Substudy Design

All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy).

Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL).

Table 9.1.7 presents the schedule of assessments for participation in the liver biopsy substudy.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	Smart Rescreening[i] (Day -28 to Day -1)	Baseline (Day -7 to Day -1)[h]	
Informed consent	X			
Medical History	X			
Physical Examination[a]	X		X	X
Height and Weight[a]	X			
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, coagulation screen, and CRP[e]	X	X	X	
Urine Tests[e]	X	X	X	
Liver Tests[e]	X	X	X	
PBMC collection for CTL baseline			X	
Von Willebrand Factor Antigen (VWF:Ag)	X			
Direct Thrombin Test			X	
PCR of vector DNA in blood, saliva, urine, semen, and stools			X	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

[b] Includes baseline hFVIII activity (chromogenic substrate assay and one-stage clotting assay), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). Sample collection on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion must be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up (Week 1-16)

Assessment	Follow-Up After BMN 270 Administration – Weeks*																
	Week 1																
	D2	D4	D8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical examination[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X		X				X
Urine Tests[b]						X				X				X				X
Liver Tests (local)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer						X				X				X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X	X	X			X		X		X		X		X		X		X
Exploratory biomarker assessments[f]						X				X				X				X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C																		X[g]
PBMC collection			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Von Willebrand Factor Antigen (VWF:Ag)						X				X				X				X
Direct Thrombin test			X												X			

* Visit windows are ± 48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.9.5.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then until at least 3 consecutive negative results are obtained. Collection and testing of semen samples must continue through Week 12, even if 3 consecutive negative samples in that compartment have already been recorded.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[g] Testing for reactivation of hepatitis B and hepatitis C at Week 16, for subjects with a past medical history of Hepatitis B or Hepatitis C prior to study entry, should be performed only in subjects who have not received therapeutic corticosteroids prior to Week 16; subjects who have received therapeutic corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.6.

Table 9.1.3: Schedule of Events – Safety Follow-Up (Week 17-32)

Assessment	Follow-Up After BMN 270 Administration – Weeks*															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight										X						
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X				X				X				X
Urine Tests[b]				X				X				X				X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer				X								X				
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X				X				X				X
Exploratory biomarker assessments[f]				X				X				X				X
Haemo-QoL-A QoL assessment												X				
AAV5 antibody titer				X				X				X				X
PBMC collection	X	X	X	X		X		X		X		X		X		X
Von Willebrand Factor Antigen (VWF:Ag)				X				X				X				X
Direct Thrombin test										X						

* Visit windows are ± 48 hours

[a] Brief physical examination should be done at all weekly visits. Refer to Section 9.7.9.5.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection for each matrix to occur until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.4: Schedule of Events – Safety Follow-Up (Week 33-52)

	Year 1 – Weeks*											
Assessment	**33**	**34**	**35**	**36**	**38**	**40**	**42**	**44**	**46**	**48**	**50**	**52**
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X
Weight[a]												X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X
Urine Tests[b]				X		X		X		X		X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X
AAV5 antibody titer				X		X		X		X		X
FVIII antibody titer				X				X				X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)		X		X				X				X
Von Willebrand Factor Antigen (VWF:Ag)				X		X		X		X		X
Direct Thrombin Test					X							X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X		X		X		X		X
Exploratory biomarker assessments[f]				X		X		X		X		X
Haemo-QoL-A QoL assessment												X

* Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52.

[a] Complete physical examination should be performed at Week 52; brief physical examination may be performed at other study visits. Refer to Section 9.7.9.5.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests,

and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.5: Schedule of Events – Safety Follow-Up (Years 2-7)

Assessment	Years 2-5*	Year 2*	Years 3-5*	Years 6-7		End of Year Visits						ETV
	Q12W	Q4W[h]	Q6W[h,j]	Q13W (Remote)[i]	Q26W	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	
						W104	W156	W208	W260	W312	W364	
Physical examination[a]	X[a]					X[a]				X	X	X
Weight[a]	X[a]					X[a]				X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X				X	X	X
Vital Signs	X					X				X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]	X				X	X				X	X	X
Urine Tests[b]	X					X						X
Liver Tests (local)[b]	X	X	X			X						X
FVIII assays (local)[c]	X	X	X			X						X
Liver Tests (central)[b]	X	X	X		X	X				X	X	X
FVIII assays (central)[d]	X	X	X		X	X				X	X	X
AAV5 antibody titer	X					X				X	X	X
FVIII antibody titer	X					X				X	X	X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)	X					X						X
Von Willebrand Factor Antigen (VWF:Ag)	X					X						X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X[e]	X[e]	X[e]		X[e]	X[e]				X[e]	X[e]	X
Exploratory biomarker assessments[g]	X					X				X	X	X
Haemo-QoL-A QoL assessment	X[f]				X[f]	X[f]				X[f]	X[f]	X

* Visit windows are ± 2 weeks for visits in Years 2-5 and ± 4 weeks for visits in Years 6-7. The Q6W visits during Years 3-5 should restart after each End of Year visit (eg, the first Q6W visit during Year 3 should be ~6 weeks after the End of Year 2 visit).

[a] Complete physical examination should be performed at the End of Year visits; brief physical examination may be performed at other study visits. Weight should be recorded at the second Q12W visit each year during Years 2-5, and at every End of Year visit during Years 2-7.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are ≥1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT ≥ 1.5x ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. During Years 2-5 of the Post-Infusion Follow-Up period, urine tests and blood, chemistry, and coagulation tests should be performed at the second Q12W visit each year and at every End of Year visit. During Years 6-7, these tests should be performed at the Q26W and End of Year visits.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) during Years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) in years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), and/or every 26 weeks (during Years 6-7) until 3 consecutive samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).

[f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at the second Q12W visit each year and at every End of Year visit. During Years 6-7, the Haemo-QoL-A assessment should be performed at the Q26W visits and the End of Year visits.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be

associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Subjects who meet the definition of treatment failure to BMN 270 therapy after Week 52 may omit the Q4W and Q6W visits during Years 2-5, and must attend only the Q12W and End of Year visits. Such subjects following the abbreviated schedule who have not yet cleared vector shedding in a fluid must still provide samples Q4W (during Year 2), Q6W (during Years 3-5), or Q26W (during Years 6-7), until vector shedding has been cleared (but do not need to do other scheduled assessments at those visit dates), either by reporting to the site to provide samples or by providing those samples to an MN professional.

[i] For every 13 week remote visits during Years 6-7, the site should contact the subject to collect data on adverse events, new or changed concomitant medications, and diary entries (FVIII usage and bleeding events).

[j] At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office. Refer to Section 9.7.9.2 for details.

Table 9.1.6: Schedule of Events – Therapeutic Corticosteroids for LT Elevations

	Steroid Treatment Period[d]											Post-Steroid Period[c]				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11[b]**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 13**
Therapeutic corticosteroids (dose in mg/day) [a]	60 mg	60 mg	40 mg	40 mg	30 mg	30 mg	20 mg	20 mg	15 mg	10 mg	5 mg					
FVIII activity testing												X	X	X	X	
Liver testing												X	X	X	X	
Hepatitis B testing[e]						X						X				X
HCV Viral Load[e]						X						X				X

[a] Therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor.

[b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

[c] After discontinuation of corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. If these assessments are already being done as part of normal study follow-up, they do not need to be duplicated.

[d] Regardless of the assessments listed in the Schedule of Assessments (Table 9.1.2, Table 9.1.3, or Table 9.1.4), subjects initiated on corticosteroids will only be required to have laboratory evaluations on a weekly basis.

[e] Should only be performed in subjects with a history of hepatitis B or hepatitis C prior to study entry.

Table 9.1.7: Schedule of Events – Liver Biopsy Substudy

	Within 28 Days Before Biopsy Day	Within 7 Days Before Biopsy Day	1 Day Before Biopsy Day [d]	Biopsy Day (BD)
Informed Consent	X			
Liver Ultrasound[a]	X			
Brief Physical Examination	X		X	X
Hematology, Coagulation, Chemistry Assessments[b]	X		X	
Liver Tests[b]	X		X	X
FibroScan		X		
Local FVIII Activity Level Assessment		X	X	X
Pre-Biopsy Consultation[c]		X		
Liver Biopsy[e]				X

[a] Liver ultrasound must be performed within 28 days prior to the scheduled biopsy, unless an ultrasound result from the prior 3 months is already available.

[b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests.

[c] Subjects will undergo a pre-biopsy consultation with the investigator (treating hematologist).

[d] Subjects may need to repeat/extend this visit if their FVIII activity levels need adjustment before the biopsy, at the discretion of the investigator (treating hematologist).

[e] Biopsy will be a percutaneous or transjugular biopsy under ultrasound guidance, performed according to the standard procedure of the institution. If only a small amount of tissue (< 2 cm) is obtained at the time of the biopsy, the subject may be asked to consent for a second pass. In this case, the original < 2 cm sample should still be retained and handled according to the instructions for handling biopsy specimens in the Laboratory Manual. Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive semen samples below the limit of detection of the test.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4.
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of < 100 x 10^9/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Liver Biopsy Substudy Inclusion and Exclusion Criteria

Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria:

1. Currently enrolled in 270-201
2. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy
3. Able to sign informed consent and comply with requirements of the substudy
4. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy:

1. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to):
   - Significant thrombocytopenia (platelet count < 100 x 10^9/L)
   - Evidence of significant ascites
   - Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.

9.3.4 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol

- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

Subjects may be considered lost to follow-up if the site has documented at least 4 attempted contacts by key research personnel to reach the subject without success in the following manner:

- 2 attempts by telephone or email (if possible); then
- If telephone/email contacts are unsuccessful, 2 attempts must be made by certified letter or by appropriate local process.

Where communication has been made by phone, this should be documented in the subject source notes.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- Any ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270, in the absence of a definitive alternate etiology for the increase
- The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion

- The detection of AAV vector DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than 52 weeks after BMN 270 infusion, as discussed in Section 9.7.9.6
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt:

- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days

9.3.5 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.6 Duration of Subject Participation

The duration of this study will be approximately 368 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 348 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical examination performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (eg, the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be discharged from the clinic unless toxicity has been observed in which case the stay in the clinic may be extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each

study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

For Cohorts 1 to 3, approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in that cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design for Cohorts 1-3

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents
- Emicizumab
- Fitusiran
- Concizumab
- Efavirenz

Subjects who begin antiretroviral therapy following BMN 270 infusion and during their participation in 270-201 must have their antiretroviral medication regimen approved by the Investigator and Medical Monitor prior to starting antiretroviral treatment.

Medications which are predominately metabolized by the liver (eg, acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52). LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

9.4.8.2.1 Therapeutic Corticosteroids

In general, therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator.

Reports of raised LTs (defined as ALT ≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

Following initiation or completion of therapeutic corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

Treatment with prednisolone will be initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.6).

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

Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. For the subjects with a past medical history of hepatitis B or hepatitis C prior to study entry, Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on a dispensing log. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data, if allowed by local SOPs.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials (or must be referenced in their institution SOPs).

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a study drug return form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

Subjects should be advised to abstain from any blood or sperm donation after BMN 270 infusion, until there is no further evidence of vector shedding from PCR analysis of samples.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.9.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1 through Table 9.1.5) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to expression of FVIII at 5 IU/dL or above, (ie, a mild severity). This is associated in natural history studies with clinically superior long term outcomes (Den Ujil, 2011).

The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic substrate assay)
- FVIII activity by one-stage clotting assay

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

The FVIII activity level in both assays and the number of subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.

FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5 but will be used in

conjunction with local lab LT assessments to monitor subject safety and need for initiation of therapeutic corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage clotting and/or chromogenic substrate assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5.

9.7.6 Exploratory Assessments

Blood samples will be collected at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

Liver biopsy samples collected as part of the exploratory substudy may also be tested to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer. Subjects participating in the substudy may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed.

On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with samples collected specifically for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Liver Biopsy Substudy

The objectives of the liver biopsy substudy are considered exploratory.

Subjects who consent to the procedure will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound exam of the liver within 28 days prior to the procedure (to ensure there is no obstruction to the liver that would interfere with the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate any histopathologic findings on the biopsy). Subjects will also undergo a pre-biopsy consultation with the investigator (treating hematologist).

Within 24 hours prior to the biopsy being performed, subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). FVIII activity levels for this purpose should be assessed at the local laboratory within 7 days before the biopsy, and again the day before the biopsy. As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".

The liver biopsy should be performed in the morning, and the biopsy procedure and follow-up care should be done according to the local standard of care. Additional details for handling the biopsy specimens are provided in the Laboratory Manual.

Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion and/or according to local guidelines.

Safety findings arising from the histopathological analysis of the biopsy sample are subject to Adverse Event reporting (Section 10), and such findings should be further assessed and followed-up as clinically appropriate and in order to safeguard the subject's ongoing medical care (this should be done in consultation with a hepatologist and/or other specialist clinicians if required). In the event fibrotic changes being observed on the biopsy sample, additional follow-up Fibroscans may be considered (with the frequency of subsequent scans at the discretion of the Investigator and/or hepatologist).

Where a biopsy has been taken for safety-related reasons (rather than as part of the liver biopsy substudy) or was available from a past procedure, the Sponsor may want to see that biopsy information to help evaluate the impact of BMN 270 on the liver. The Sponsor may

request that slides from a liver biopsy not performed as part of the substudy be made available for additional histopathological review.

9.7.9 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.9.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5.

9.7.9.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. The scheduled clinical laboratory tests are listed in Table 9.7.9.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.9.2.1: Clinical Laboratory Tests

Blood Chemistry	Haematology	Urine Tests	Other
Albumin	Haemoglobin	Appearance	CRP
BUN	Haematocrit	Color	
Calcium	WBC count	pH	**Coagulation Screen including:**
Chloride	RBC count	Specific gravity	
Total cholesterol	Platelet count	Ketones	APTT
CO_2	Differential cell count	Protein	PT/INR

CPK		Glucose	TT
Creatinine		Bilirubin	
Glucose		Nitrite	**Other Tests:**
Phosphorus		Urobilinogen	ABO blood typing*
Potassium		Haemoglobin	
Total protein			
Sodium			
Uric Acid			

BUN, blood urea nitrogen; CO_2, carbon dioxide; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

* ABO blood typing assessment should be completed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study).

At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

9.7.9.3 Liver and Hepatitis Testing

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active hepatitis B or hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing hepatitis B or hepatitis C infection is exclusionary. Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received as part of their medical history assessment at Screening.

Subjects with a history of hepatitis B or hepatitis C infection prior to study entry will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

A liver ultrasound and LTs at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

Liver tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. At each time point, the following LTs should be assessed:

Table 9.7.9.3.1: Liver Tests

Liver Tests (LTs)			
Alkaline Phosphatase	AST (SGOT)	Total Bilirubin	LDH
ALT (SGPT)	Direct Bilirubin	GGT	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase

Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

9.7.9.4 HIV Testing

HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.

9.7.9.5 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every 15 minutes

(± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and at the End of Years visits thereafter. A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems.

At other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, dermatologic, musculoskeletal, and gastrointestinal assessments. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

Height will be recorded at Screening only. Weight will be recorded at Screening, at Weeks 26 and 52 during Year 1, and then at the second Q12W visit each year during Years 2-5 and at every End of Year visit during Years 2-7.

9.7.9.6 Vector Shedding

Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014); (Manno, 2006); (Schenk-Braat, 2007); (Croteau, 2004). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid but not in motile sperm (Manno, 2006). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016).

During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of vector shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive samples below the limit of detection of the test are documented (or upon consultation between the Investigator and Medical Monitor). Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule (refer to Section 12.5.3 but who have not cleared vector shedding from all fluids must still provide samples for assessment until vector shedding has cleared: every 4 weeks (during Year 2), every 6 weeks (during Years 3-5), or every 26 weeks (during Years 6-7), either by reporting to the site to provide samples or by providing those samples to an MN professional.

Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive semen samples below the limit of detection (upon consultation between the Investigator and Medical Monitor).

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (eg, sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.1.1.1 Bleeding and Suspected Bleeding Events

All bleeding events and suspected bleeding events, regardless of the need for exogenous FVIII therapy as treatment, should be captured in subject diaries and recorded on the designated bleeding eCRF. Bleeding events and suspected bleeding events should not be reported as adverse events, with the following exception:

- All bleeding events and suspected bleeding events which meet one or more of the criteria for being serious (refer to Section 10.2) should be reported as serious adverse events (whether or not they are bleeding events that are normal sequelae of hemophilia, and whether or not they required exogenous FVIII as treatment).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening
- *Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.*
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (eg, anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of ALT ≥1.5x ULN, regardless of whether that triggers an initiation or modification of corticosteroid treatment
- Events meeting the criteria for Hy's law (ALT or AST elevation ≥ 3x ULN plus total bilirubin ≥ 2x ULN)
- Thromboembolic event
- Development of anti-FVIII inhibitory antibodies (inhibitors)

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 7 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.1.1.1.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.1.1.1 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.1.1.1. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE (or SAE, as appropriate) eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. Events that change in severity necessitate the recording of an additional AE. AEs that do not have a change in severity should be recorded only once on the eCRF.

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE or EOSI should be reported as such, and recorded in the AE eCRF. Any laboratory result abnormality of CTCAE Grade 4 or 5 should be recorded as an SAE in the AE eCRF, unless the abnormal laboratory results has been reported or captured as part of an underlying diagnosis.

A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Requiring a change in concomitant therapy (eg, addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless there is an impact to clinical outcomes (eg, increased rate of bleeding, worsening of joint disease).

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.1.1.1).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated procedure or assessment
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Insert an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug or FVIII replacement therapy.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record “Unexplained Death” or “Death of Unknown Cause” on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the

Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up eCRF. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any

events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax:
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI, MA MB BChir MSc
Address: Biomarin (UK) Ltd.
10 Bloomsbury Way
London WC1A 2SL
Phone PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic substrate assay and the one-stage clotting assay are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (+ 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, hepatitis B, hepatitis C, and HIV.
    - Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received. Any prior pharmacokinetics information obtained while the subject was receiving prophylactic or on-demand FVIII therapy prior to the study should also be collected. For subjects who have already enrolled in 270-201, this information should be collected at the next regularly scheduled study visit (or at least prior to the subject's completion of study participation).
- Complete Physical Examination
- Height and weight
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic substrate (plasma)
    - Baseline hFVIII activity level – one-stage clotting assay

- hFVIII coagulation activity exploratory assay
- hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- hFVIII total antibody assay
- hFVIII antigen (ELISA)

- Blood sample for AAV5 Assays
  - AAV5 antibody titer
  - AAV5 transduction inhibition assay
- Screen for hepatitis B, hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1)
- Von Willebrand Factor Antigen (VWF:Ag)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- Blood sample for AAV5 Assays
  - AAV5 antibody titer

- AAV5 transduction inhibition assay

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1)
- PBMC collection for CTL baseline
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- BMN 270 Infusion

- Vital Signs
    - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Once per week (Weeks 1 through 16)

The following procedures will be performed at one visit per week from Weeks 1 through 16:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- Samples for FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
    - Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16

- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- PBMC collection

12.5.2 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only) – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Liver Tests (refer to Table 9.7.9.3.1) (Day 4 only) – central laboratory assessment
- Liver Tests (refer to Table 9.7.9.3.1) (Day 4 only) – local laboratory assessment
- FVIII activity level (Day 4 only) – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

12.5.3 Week 1 – Day 8

On Day 8, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Direct Thrombin test

- Haemo-QoL-A QoL assessment

12.5.4 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1) (not assessed at Week 14)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2)
    - Collection to occur until at least 3 consecutive negative results are obtained. Semen samples should continue to be collected and tested through Week 12, even if 3 consecutive negative results in that compartment have been recorded prior to that timepoint.

12.5.5 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedures will be performed:

- Urine Tests (refer to Table 9.7.9.2.1)
- VWF:Ag
- FVIII antibody titer
- Exploratory biomarker assessments

12.5.6 Week 16

At Week 16, the following procedures will be performed:

- Test for hepatitis B and hepatitis C reactivation (in subjects with a history of hepatitis B or hepatitis C infection prior to study entry)
    - Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

12.5.7 Weeks 8 and 16

At Weeks 8 and 16, the following procedures will be performed:

- AAV5 antibody titer

12.5.8 Weeks 2, 3, 4, and 16

At Weeks 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.5.9 Week 13

At Week 13, the following procedure will be performed:

- Direct Thrombin test

12.6 Safety Follow-Up – Weeks 17-36

After the Post-Infusion Follow-Up visits are complete, subjects will return to the study site for Safety Follow-Up visits from Weeks 17 through Week 36 (± 48 hours), when the following procedures will be completed:

12.6.1 Once per week (Weeks 17 through 36)

Once per week from Week 17 through Week 36, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

12.6.2 Once per week (Weeks 17 through 20)

Once per week from Week 17 through Week 20, the following procedures will be performed:

- PBMC collection

12.6.3 Every 2 weeks (Weeks 21 through 36)

Every 2 weeks (Weeks 22, 24, 26, 28, 30, 32, 34, and 36), the following procedures will be performed:

- PBMC collection

12.6.4 Every 4 Weeks

Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- VWF:Ag
- AAV5 antibody titer
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.5 Every 8 Weeks

Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed:

- FVIII antibody titer

12.6.6 Week 26

At Week 26, the following procedure will be performed:

- Direct Thrombin test
- Weight

12.6.7 Week 28

At Week 28, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Weeks 37-52

Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:

12.7.1 Once per visit

At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

12.7.2 Every 4 Weeks

Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- AAV5 antibody titer
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.7.3 Every 8 Weeks

Every 8 weeks (Weeks 44 and 52), the following procedure will be performed:

- PBMC collection
- FVIII antibody titer

12.7.4 Week 38 and 52

At Week 38 and Week 52, the following procedure will be performed:

- Direct Thrombin test

12.7.5 Week 52

At Week 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment
- Weight

12.8 Safety Follow-Up – Years 2-5

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52 of the study by attending only the Q12W and End of Year visits during Years 2-5.

Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from a fluid must still provide samples for assessment every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until vector shedding has cleared (but do not have to perform other scheduled assessments at those study visits).

At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study.

During Years 2-5 of Safety Follow-up, the following procedures will be completed:

12.8.1 Year 2 – Every 4 Weeks (not required for treatment failure)

During Year 2, every 4 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Year 2 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks during Year 2 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).
    - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks during Year 2 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.2 Years 3-5 – Every 6 Weeks (not required for treatment failure)

During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

- FVIII coagulation activity exploratory assay
- Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
- FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
  - Sample testing during Years 3-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52 and/or Year 2. Subjects who have not had 3 consecutive negative semen samples by the end of Year 2 should continue to have PCR testing of semen every 6 weeks during Years 3-5 until 3 consecutive samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).
  - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from a fluid, must still provide samples of that fluid for assessment every 6 weeks during Years 3-5 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.3 Years 2-5 – Every 12 Weeks and End of Year Visits (required for all subjects)

During Years 2-5, subjects will be asked to return to the study site for visits at the following study weeks (±2 weeks):

- Year 2 – Week 64, Week 76, Week 88, Week 100, Week 104
- Year 3 – Week 116, Week 128, Week 140, Week 152, Week 156
- Year 4 – Week 168, Week 180, Week 192, Week 204, Week 208
- Year 5 – Week 220, Week 232, Week 244, Week 256, Week 260

For each of these years, the last study visit listed (Week 104, Week 156, Week 208, and Week 260) will serve as an End of Year visit.

At the every 12 week and End of Year visits, the following procedures will be performed:

- Brief physical examination (at every 12 week visits) or complete physical examination (at End of Year visits)
- Weight (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
    - ABO blood typing assessment should be performed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study)
- Urine Tests (refer to Table 9.7.9.2.1)
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection

- VWF:Ag
- Exploratory biomarker assessments
- Haemo-QoL-A QOL assessment (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Years 2-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).

12.9 Safety Follow-Up – Years 6-7

If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.

During Years 6-7 of Safety Follow-up, the following procedures will be completed:

12.9.1 Years 6-7 – Every 13 Weeks

Every 13 weeks (±4 weeks) during Years 6-7, the site should contact the subject remotely to collect the following data:

- Adverse events
- New or changed concomitant medications
- Diary entries (FVIII usage and bleeding events)

12.9.2 Years 6-7 – Every 26 Weeks and End of Year Visits

During Years 6-7, the following assessments will be performed at a site visit every 26 weeks (±4 weeks) (Week 286 and Week 338) and at the End of Year Visits (±4 weeks) (Week 312 and Week 364):

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Complete Physical Examination (Week 312 and Week 364 only)
- Weight (Week 312 and Week 364 only)
- Vital signs (Week 312 and Week 364 only)

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- AAV5 antibody titer (Week 312 and Week 364 only)
- FVIII antibody titer (Week 312 and Week 364 only)
- Exploratory biomarker assessments (Week 312 and Week 364 only)
- Haemo-QoL-A QOL assessment
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Years 6-7 is not required if at least 3 consecutive samples are below the limit of detection as of the end of Year 5.

12.10 Liver Biopsy Substudy - Years 1-5

For the optional liver biopsy substudy (refer to Table 9.1.7), the following procedures will be performed at the following times:

- Within 28 days before Biopsy Day
    - Informed consent
    - Liver ultrasound
        - Liver ultrasound does not need to be performed if an ultrasound result from the prior 3 months is already available
    - Brief physical examination

- Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1)

- Within 7 Days before Biopsy Day
  - FibroScan
  - Local laboratory FVIII activity level assessment
  - Pre-biopsy consultation with investigator (treating hematologist). This may need to be repeated/extended if the subject's FVIII activity levels need adjustment before the biopsy, at the discretion of the investigator (treating hematologist).
- 1 Day before Biopsy Day
  - Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.9.2.1)
  - Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
  - Brief physical examination
  - Local laboratory FVIII activity level assessment
    - Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".
- Biopsy Day
  - Brief physical examination
  - Local laboratory FVIII activity level assessment (performed pre-biopsy)
    - Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".

- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment (pre-biopsy)
- Liver Biopsy (refer to Laboratory Manual for guidelines for biopsy procedure and handling of biopsy samples)
  - If only a small amount of tissue is obtained at the time of the biopsy, the subject may be asked to consent for a second pass.

Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion.

12.11 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 364 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1)
- Urine Tests (refer to Table 9.7.9.2.1)
- Liver Tests (refer to Table 9.7.9.3.1) – local laboratory assessment
- FVIII activity level – local laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment
- FVIII Assays – central laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
  - FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if at least 3 consecutive samples are below the limit of detection prior to the ETV.
- Exploratory biomarker assessment
- Haemo-QoL-A QOL assessment

12.12 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 364). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay as measured by the central laboratory at 16 weeks following study treatment. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Liver Biopsy Substudy Analysis

A separate report presenting and discussing analyses of the exploratory objectives for the optional liver biopsy substudy will be prepared.

14.4 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.5 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by a one-stage clotting assay and/or chromogenic substrate assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.6 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.7 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.8 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.9 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COSTS, COMPENSATION, AND SUBJECT INJURY

BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries. If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study subject's primary disease or any concurrent disease and that are unrelated to this study.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked.

Upon completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas AND to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572 and/or principles of ICH E6 R2 GCP, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and/or ICH E6 R2 Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/ IEC review and approval in 21 CFR Part 56 and/or ICH E6 R2 Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH E6 R2 Section 4.11.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH E6 R2 Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH E6 R2 Section 4.9.7.
- The IRB/IEC/REB complies with the requirements of 21 CFR Part 56, ICH E6 R2 Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201 Amendment 9

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including ICH E6 R2, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name _____

Accepted for the Sponsor:

DocuSigned by:
PI
Signing Reason: I approve this document
Signing Time: 22-Jun-2020 | 8:49 AM PDT
D81F76E32EA74F83864C63F8E75F1EBE

Medical Monitor Signature Date

Printed name: PI, MA MB BChir MSc, PI Clinical Science___

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See page 2-4). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Revision	Rationale
2/Synopsis (Study Rationale)	The vector genome figure was updated.	10
2/Synopsis (Study Design and Plan)	At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.	2
2/Synopsis (Inclusion Criteria)	Individuals eligible to participate in this study must meet all of the following criteria: 6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive ~~negative~~ semen samples below the limit of detection of the test.	7
2/Synopsis (Criteria for Evaluation)	No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52) during Year 1. LTs will be monitored every 4 weeks during Year 2, ~~and then~~ every 6 weeks for Years 3-5, and every 26 weeks for Years 6-7 post-dose in the safety extension; the frequency and duration of LT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.	1
2/Synopsis (Statistical Methods)	The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay at 16 weeks following study treatment. ~~FVIII activity will be assessed weekly during the study period.~~	13

Section No./Title	Revision	Rationale
5.2/Ethical Conduct of Study	Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. ~~Each subject will provide written, informed consent before any study-related tests or evaluations are performed.~~ Each subject will provide written informed consent before any study-related tests or evaluations are performed, as well as when updates to the ICF are made. Where it is not feasible for the Investigator to receive the signed, written ICF from the subject prior to beginning new or changed study-related procedures contained in such an ICF update (for example, due to COVID-19-related restrictions), the Investigator will ask the subject to verbally confirm during an informed consent interview that the subject has signed and dated the ICF. The Investigator should document this verbal confirmation on the Investigator's copy of the ICF and, when it is possible to do so, receive the informed consent signed by the subject and archive the original(s) in the record file of the subject.	8
7.3/Study Rationale	The vector genome figure was updated.	10
7.3.1/Liver Biopsy Substudy Rationale	Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or the variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown. Finally, a call to incorporate liver biopsy sub-studies into gene therapy trials for hemophilia has been issued by medical and scientific leaders in the field to help illuminate these and other questions (National Hemophilia Foundation, 2019).	13
7.4/Summary of Overall Risks and Benefits	~~The majority of subjects in the ongoing 270-201 clinical study who have received 4E13 or 6E13 vg/kg doses of BMN 270 have had Grade 1 asymptomatic elevations in ALT. For most subjects, the elevations have reached only slightly above the upper limit of normal (ULN). Based on the effectiveness of transient oral corticosteroid used to suppress a presumed Class 1 (cytotoxic T-cell) response in prior studies with hepatic transduction with AAV vectors (Mingozzi, 2013), subjects were treated with 7-32 weeks of oral corticosteroids prophylactically or in response to the elevations in ALT to ensure preservation of the transduced hepatocytes. Using this approach, no sustained loss of FVIII activity has been observed in subjects with ALT elevations, consistent with maintaining a high level of hepatocyte function. Moreover, the rise in ALT levels were not accompanied by significant or lasting aberrations in other liver tests such as AST, bilirubin or albumin, indicating that extent of toxicity is limited. There has been one HIV-positive subject in the ongoing 270-302 clinical study who experienced Grade 3~~	12

Section No./Title	Revision	Rationale
	~~asymptomatic elevations in ALT and AST, which has been attributed to an interaction between one or more of his antiretroviral therapy medications and/or unsuspected underlying hepatic disease with BMN 270. In addition, there has been one subject with Gilbert's syndrome in the ongoing 270-301 clinical study who has experienced Grade 3 asymptomatic elevations in ALT and AST. These cases have led to the exclusion of subsequent HIV-positive subjects and requirement of liver tests at Screening that are <1.25 times the upper limit of the normal range in the ongoing 270-301 and 270-302 clinical studies. Of note, two HIV-positive subjects in 270-301 and one presumed Gilbert's syndrome subject in 270-201 have received BMN 270 without experiencing any elevations in ALT to date. Overall, the literature and clinical experience with BMN 270 suggests that transient elevations in liver enzymes are expected following AAV-based gene therapy for the treatment for hemophilia A or B without any long-term concerns of hepatic injury (Manno, 2006; Nathwani, 2011; George, 2016; Miesbach, 2016; Pasi, 2016).~~ BMN 270 has an acceptable safety and tolerability profile that supports a positive benefit-risk assessment. Single infusions have been generally well tolerated by treated subjects across all investigated doses. All subjects have successfully completed their full-dose infusion of BMN 270, with no infusions requiring permanent termination prior to completion due to AEs. No deaths have been reported in any of the BMN 270 studies, and no participants discontinued from studies as a result of an AE. Frequency of adverse events decreased over time with no delayed adverse drug reactions. Infusion reactions associated with BMN 270 administration included symptoms such as maculopapular rash, urticaria, nausea, diarrhea, watery eyes, rigors, chills, myalgia, fever, tachycardia and hypotension emerging within 24 hours of receiving BMN 270. All of these events subsided without clinical sequela within 48 hours following medical management Infusion-related reactions were effectively mitigated by managing infusion rate and medications. Transient, asymptomatic ALT elevation (grade 1 to 3 in severity) was observed in most subjects administered BMN 270 shortly after dosing, with no symptoms or sequelae suggestive of clinically significant hepatocyte injury or liver dysfunction. In almost all subjects, ALT elevations decreased quickly following corticosteroid treatment. There were differences in the use of corticosteroids across studies. Subjects in 270-201, who received corticosteroids an average of 8 weeks earlier following BMN 270 infusion than the mITT population in 270-301, were more likely to avoid a significant decline in FVIII activity concurrently with an ALT elevation, and saw a more robust recovery of FVIII activity upon the first use of corticosteroids, than did the subjects in the mITT population in 270-301. Despite the clinical response to steroids, no associations between safety parameters (transient ALT rises), or efficacy as measured by FVIII activity levels were found to be temporally associated with anti-AAV5 antibody or cellular immune responses.	

Section No./Title	Revision	Rationale
	At the highest dose tested in 270-201 (6E13 vg/kg), the majority of subjects achieved FVIII levels above 50 IU/dL at 52 weeks post-infusion. Subjects in that cohort also reported markedly decreased bleeding compared with pre-study rates and the ability to discontinue prophylactic FVIII infusions. Subjects at all dose levels continue to be followed. In 270-301, an interim analysis has shown increased FVIII activity in the majority of subjects to mild HA or normal levels at 26 weeks post-infusion, also with markedly decreased bleeding compared with pre-study rates and the ability to discontinue prophylactic FVIII infusions. All subjects who will be included in the final analysis have been dosed with 6E13 vg/kg and continue to be followed. The current data available ~~for BMN 270 does not yet permit comprehensive assessment of the~~ has shown an established positive benefit~~:~~-risk profile ~~of this investigational drug~~for BMN 270 at the 6E13 vg/kg dosing level. Given the monitoring measures in place in the clinical protocol(s) to minimize the risk to subjects participating in the existing studies, the identified risks are justified by the anticipated benefits that may be afforded to subjects. Each subject in 270-~~201~~301 will have a comprehensive surveillance plan that monitors LTs during the study, and elevations in ~~LT~~LTs will be addressed according to the guidelines set forth in the protocol. Safety will be assessed by adverse event reporting and clinical laboratory assessments.	
9.1/Overall Study Design and Plan	At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.	2
Table 9.1.5/SOE (Years 2-7)	Table 9.1.5 was updated to reflect changes made elsewhere in the protocol.	1, 2
Table 9.1.5 Notes	* Visit windows are ± 2 weeks for visits in Years 2-5 and ± 4 weeks for visits in Years 6-7. The Q6W visits during Years 3-5 should restart after each End of Year visit (eg, the first Q6W visit during Year 3 should be ~6 weeks after the End of Year 2 visit).	1, 2, 7, 13

Section No./Title	Revision	Rationale
	[a] Complete physical examination should be performed at the End of Year visits; brief physical examination may be performed at other study visits. Weight should be recorded at the second Q12W visit each year during Years 2-5, and at every End of Year visit during Years 2-~~5~~7. [b] Refer to Table 9.7.9.2.1 for laboratory assessments to be included, and to Table 9.7.9.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are ≥ 1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT ≥ 1.5x ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. During Years 2-5 of the Post-Infusion Follow-Up period, urine tests and blood, chemistry, and coagulation tests should be performed at the second Q12W visit each year and at every End of Year visit. During Years 6-7, these tests should be performed at the Q26W and End of Year visits. [e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2~~) or~~), every 6 weeks (during Years 3-5), and/or every 26 weeks (during Years 6-7) until 3 consecutive ~~negative~~ samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor). [f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at the second Q12W visit each year and at every End of Year visit. During Years 6-7, the Haemo-QoL-A assessment should be performed at the Q26W visits and the End of Year visits. [h] Subjects who meet the definition of treatment failure to BMN 270 therapy after Week 52 may omit the Q4W and Q6W visits during Years 2-5, and must attend only the Q12W and End of Year visits. Such subjects following the abbreviated schedule who have not yet cleared vector shedding in ~~all fluids~~a fluid must still provide samples Q4W (during Year 2~~) or~~), Q6W (during Years 3-5~~)~~), or Q26W (during Years 6-7), until vector shedding has been cleared (but do not need to do other scheduled assessments at those visit dates), either by reporting to the site to provide samples or by providing those samples to an MN professional.	

Section No./Title	Revision	Rationale
	[i] For every 13 week remote visits during Years 6-7, the site should contact the subject to collect data on adverse events, new or changed concomitant medications, and diary entries (FVIII usage and bleeding events). [j] At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office. Refer to Section 9.7.9.2~~).~~ for details.	
9.3.1/Inclusion Criteria	Individuals eligible to participate in this study must meet all of the following criteria: 6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive ~~negative~~ semen samples below the limit of detection of the test.	7
9.3.4/Removal of Subjects from Treatment or Assessment	Subjects may be considered lost to follow-up if the site has documented at least 4 attempted contacts by key research personnel to reach the subject without success in the following manner: • 2 attempts by telephone or email (if possible); then • If telephone/email contacts are unsuccessful, 2 attempts must be made by certified letter or by appropriate local process. Where communication has been made by phone, this should be documented in the subject source notes.	9
9.3.6/Duration of Subject Participation	The duration of this study will be approximately ~~264~~368 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and ~~244~~348 weeks of Safety Follow-Up.	1
9.4.8/Prior and Concomitant Medications	The following medications are prohibited starting 30 days before Screening: • ~~Lamivudine~~	3
9.7.8/Liver Biopsy Substudy	Where a biopsy has been taken for safety-related reasons (rather than as part of the liver biopsy substudy) or was available from a past procedure, the Sponsor may want to see that biopsy information to help evaluate the impact of BMN 270 on the liver. The Sponsor may request that slides from a liver biopsy not performed as part of the substudy be made available for additional histopathological review.	6
Table 9.7.9.2.1/Clinical Laboratory Tests	The ABO blood typing test has been listed as an "other test" rather than as part of the hematology panel.	13

Section No./Title	Revision	Rationale
Section 9.7.9.2/Clinical Laboratory Assessments	At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study. The Sponsor may select a healthcare company that will be responsible for providing MN services for participating sites (the MN vendor). The MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site.	2
9.7.9.5/Vital Signs, Physical Exams and Other Observations Related to Safety	Height will be recorded at Screening only. Weight will be recorded at Screening, at Weeks 26 and 52 during Year 1, and then at the second Q12W visit each year during Years 2-5 and at every End of ~~Years~~Year visit during Years 2-~~5~~7.	1
9.7.9.6/Vector Shedding	Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative samples below the limit of detection of the test are documented (or upon consultation between the Investigator and Medical Monitor). Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule (refer to Section 12.5.3 but who have not cleared vector shedding from all fluids must still provide samples for assessment until vector shedding has cleared: every 4 weeks (during Year 2), ~~or every 6 weeks (during Years 3-5).~~every 6 weeks (during Years 3-5), or every 26 weeks (during Years 6-7), either by reporting to the site to provide samples or by providing those samples to an MN professional. Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive ~~negative~~ semen samples below the limit of detection (upon consultation between the Investigator and Medical Monitor).	1, 7
10.2.1/Events of Special Interest	The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality: • Events meeting the criteria for Hy's law (ALT or AST elevation ≥ 3x ULN plus total bilirubin ≥ 2x ULN)	4, 5

Section No./Title	Revision	Rationale
	• Development of anti-FVIII inhibitory antibodies (inhibitors)	
10.4.1.4/Abnormal Laboratory Values	Any laboratory result abnormality fulfilling the criteria for a SAE or EOSI should be reported as such, and recorded in the AE eCRF. Any laboratory result abnormality of CTCAE Grade 4 or 5 should be recorded as an SAE in the AE eCRF, unless the abnormal laboratory results has been reported or captured as part of an underlying diagnosis. ... For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless ~~signs of worsening are observed.~~there is an impact to clinical outcomes (eg, increased rate of bleeding, worsening of joint disease).	13
12.8/Safety Follow-Up Years 2-5	At applicable sites, certain study assessments designated in the Schedule of Events may be performed by a mobile nursing (MN) professional at the subject's home or another suitable location, such as their school or office, to improve access and convenience for subjects participating in the study.	2
12.8.2/Years 3-5 – Every 6 Weeks	During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed: • PCR of vector DNA in blood, saliva, urine, semen, and stools (if required) o Sample testing during Years 3-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52 and/or Year 2. Subjects who have not had 3 consecutive negative semen samples by ~~Week 52~~the end of Year 2 should continue to have PCR testing of semen every 6 weeks during Years 3-5 until 3 consecutive ~~negative~~ samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).	7
12.8.3/Years 2-5 Every 12 Weeks and End of Year Visits	During Years 2-5, subjects will be asked to return to the study site for visits at the following study weeks (± 2 weeks): • Year 2 – Week 64, Week 76, Week 88, Week 100, Week 104 • Year 3 – Week 116, Week 128, Week 140, Week 152, Week 156 • Year 4 – Week 168, Week 180, Week 192, Week 204, Week 208 • Year 5 – Week 220, Week 232, Week 244, Week 256, Week 260 At the every 12 week and End of Year visits, the following procedures will be performed:	7, 13

Section No./Title	Revision	Rationale
	• PCR of vector DNA in blood, saliva, urine, semen, and stools (if required) ○ Sample testing during Years 2-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive ~~negative~~ samples below the limit of detection are documented (or upon consultation between the Investigator and Medical Monitor).	
12.9/Safety Follow-Up Years 6-7	If the investigator at a participating site determines that MN services are appropriate for a subject and the subject gives written informed consent to participate in MN visits, the MN network will communicate with the subject and the subject's site. During Years 6-7 of Safety Follow-up, the following procedures will be completed:	1
12.9.1/Years 6-7 – Every 13 Weeks	Every 13 weeks (± 4 weeks) during Years 6-7, the site should contact the subject remotely to collect the following data: • Adverse events • New or changed concomitant medications • Diary entries (FVIII usage and bleeding events)	1
12.9.2/Years 6-7 – Every 26 Weeks and End of Year Visits	During Years 6-7, the following assessments will be performed at a site visit every 26 weeks (± 4 weeks) (Week 286 and Week 338) and at the End of Year Visits (± 4 weeks) (Week 312 and Week 364): • Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) • Complete Physical Examination (Week 312 and Week 364 only) • Weight (Week 312 and Week 364 only) • Vital signs (Week 312 and Week 364 only) • Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.9.2.1) • Liver Tests (refer to Table 9.7.9.3.1) – central laboratory assessment ○ LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII Assays – central laboratory assessment	1

Section No./Title	Revision	Rationale
	○ FVIII activity level (one-stage clotting assay) ○ FVIII activity level (chromogenic substrate assay) ○ FVIII coagulation activity exploratory assay ○ Bethesda assay (with Nijmegen modification) for FVIII inhibitor level ○ FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. • AAV5 antibody titer (Week 312 and Week 364 only) • FVIII antibody titer (Week 312 and Week 364 only) • Exploratory biomarker assessments (Week 312 and Week 364 only) • Haemo-QoL-A QOL assessment • PCR of vector DNA in blood, saliva, urine, semen, and stools (if required) ○ Sample testing during Years 6-7 is not required if at least 3 consecutive samples are below the limit of detection as of the end of Year 5.	
12.11/ETV	If a subject leaves the study prior to the Week ~~260~~364 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done: • PCR of vector DNA in blood, saliva, urine, semen, and stools ○ Sample testing at the ETV is not required if at least 3 consecutive samples are ~~clear during the Post-Infusion Follow-Up period~~below the limit of detection prior to the ETV.	1, 7
12.12/End of Study	The study will end after the last subject completes the last Safety Follow-Up visit (Week ~~260~~364).	1
14.2/Primary and Secondary Efficacy Analysis	Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay as	13

Section No./Title	Revision	Rationale
	measured by the central laboratory at 16 weeks following study treatment. ~~hFVIII activity will be assessed weekly during the study period.~~	
16/Costs, Compensation and Subject Injury	~~There will be no charge to study subjects to be in this study.~~ BioMarin will pay ~~all~~the full costs of the study-related tests, procedures, and treatments ~~that are part of~~set forth in this ~~study~~protocol. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the reasonable cost of travel for study-related visits~~. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease that are not part of this study. Costs associated~~ in accordance with ~~hospitalizations, tests,~~BioMarin's travel and ~~treatments should be billed and collected in the way that such costs are usually billed and collected~~reimbursement policy. The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. ~~The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will~~BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures~~, if these costs are not covered by health insurance or another third party that usually pays these costs.~~. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries ~~without prior recourse to third party payer billing.~~ If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study subject's primary disease or any concurrent disease and that are unrelated to this study.	11
21/References	~~George, LA, Sullivan, S, Teitel, J, Cuker, A et al. Preliminary results of a phase 1/2 trial of SPK-9001, a hyperactive FIX variant delivered by a novel capsid, demonstrate consistent factor IX activity levels at the lowest dose cohort. Haemophilia 22[Suppl. 4], 151-152. 2016.~~ ~~Mingozzi, F and High, KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood 122[1], 23-36. 2013.~~ National Hemophilia Foundation. MASAC recommendation for liver biopsies in gene therapy trials for hemophilia. Available at https://hemophilia.org/sites/default/files/ document/files/256.pdf. 2019. MASAC Recommendation #256. Last accessed 5 June 2020.	13

Section No./Title	Revision	Rationale
	~~Pasi, J, Wong, W, Rangarajan, S, Wilde, J et al. Interim results of an open label, phase 1/2 study of BMN 270, an AAV5-FVIII gene transfer in severe hemophilia A. Haemophilia 22[Suppl. 4], 151-152. 2016.~~	

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI , MA MB BChir MSc BioMarin (UK) Ltd. 10 Bloomsbury Way London, WC1A 2SL
Duration of Subject Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015
Date of Amendment 4:	02 September 2016
Date of Amendment 5:	14 February 2017
Date of Amendment 6:	21 December 2017
Date of Amendment 7:	10 October 2018
Date of Amendment 8:	31 January 2019

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 8

Date: 31 January 2019

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 8 to the 270-201 protocol is provided below.

1. An optional liver biopsy substudy has been added to the protocol.

Rationale: BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter. It was designed to direct long term FVIII transgene expression within the liver as measured by an increase in circulating levels of hFVIII and produce less frequent episodes of bleeding and the need for exogenous FVIII infusions. Health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Inter-subject variability in post-therapy hFVIII levels has been observed. Neither the reasons for the variations in response to FVIII gene therapy nor the effects on hepatic tissue structure and function are known. The purpose of this exploratory substudy is to provide a better understanding of the variation in hFVIII levels observed after gene therapy by direct examination of liver tissue for any pathologic alterations within the liver or alterations in hepatocyte structure and to characterize the transduced gene form and distribution.

2. Assessment of the Direct Thrombin Activity test has been removed from the Year 2-5 follow-up visits.

Rationale: Based on the lack of correlation observed between FVIII activity levels and Direct Thrombin Activity results in 270-201 to date, it has been determined that samples for this exploratory test will no longer be collected during long-term follow-up.

3. Minor administrative changes have been made for consistency and clarity.

Specific changes included in this amendment, including the Synopsis, since Amendment 7 (approved 10 October 2018) are outlined in Section 24.

2 SYNOPSIS

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TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and

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maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 1).

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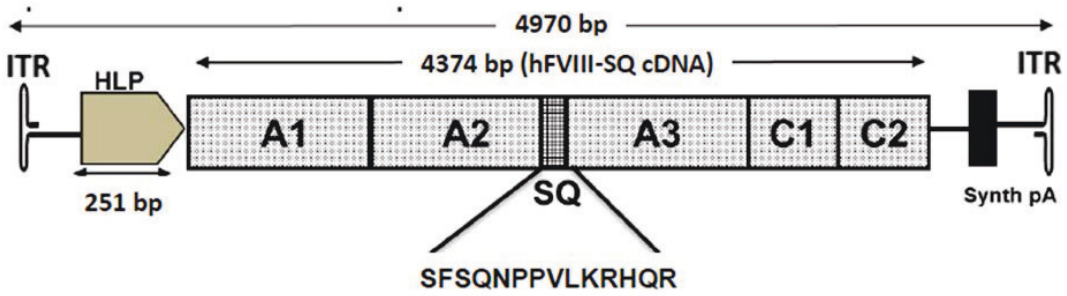
Figure 1. hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

Liver Biopsy Substudy Rationale

The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms, and it is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans.

Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations

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or variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown.

The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to circularized genomes, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.

OBJECTIVES:

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

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 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)

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STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)

Cohort 2: 2E13 vg per kilogram, iv

Cohort 3: 6E13 vg per kilogram, iv

Cohort 4: 4E13 vg per kilogram, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in non-human primates.

Cohorts 1-3

The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the one-stage clotting and chromogenic substrate assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

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If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator. Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

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Liver Biopsy Substudy Design

All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy).

Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL).

NUMBER OF SUBJECTS PLANNED:

Up to 15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months

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6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period

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13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria:

1. Currently enrolled in 270-201
2. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy
3. Able to sign informed consent and comply with requirements of the substudy
4. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy:

1. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to):
   - Significant thrombocytopenia (platelet count < 100 x 10^9/L)
   - Evidence of significant ascites
   - Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

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REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous infusion.

CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver tests (LTs, including ALT, AST, GGT, LDH, bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52) during Year 1. LTs will be monitored every 4 weeks during Year 2, and then every 6 weeks for Years 3-5 post-dose in the safety extension; the frequency and duration of LT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

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Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage clotting and/or chromogenic substrate assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

Liver Biopsy Substudy:

The following exploratory assessments will be performed as part of the optional liver biopsy substudy:

- Morphologic/pathogenic changes after FVIII gene transduction or any change that may be associated with sustained ALT rise
- Determine quantities of liver FVIII-SQ DNA/RNA
- Determine forms of vector DNA in liver at the time of biopsy
- Determine percentage of hepatocytes expressing FVIII protein
- Determine percentage of hepatocytes staining positive for vector DNA
- Determine hepatic liver transcriptome at single nuclei level if sufficient material is obtained
- Identify the forms of vector DNA in the liver
- Examination of potential stress inducing cellular pathways
- Other exploratory assessment to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at $\geq$ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay at 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**
BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates. Analysis of neutralizing antibody response and other immunological parameters as well as vector shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.		

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
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
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee

IND	Investigational New Drug (application)
INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LT	liver test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen

Definition of Terms:

Investigational Product (IP):

“A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use” (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms “IP” and “study drug” may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver tests (LTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman, 2013, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay, 2012, Blood).

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2013, Haemophilia.); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This

nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in

the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani, 2006, Blood). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Engl.J.Med.), (Nathwani, 2014, N.Engl.J.Med.). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola, 2014, J.Hepatol.). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Mannucci, 2001, N.Engl.J.Med.). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

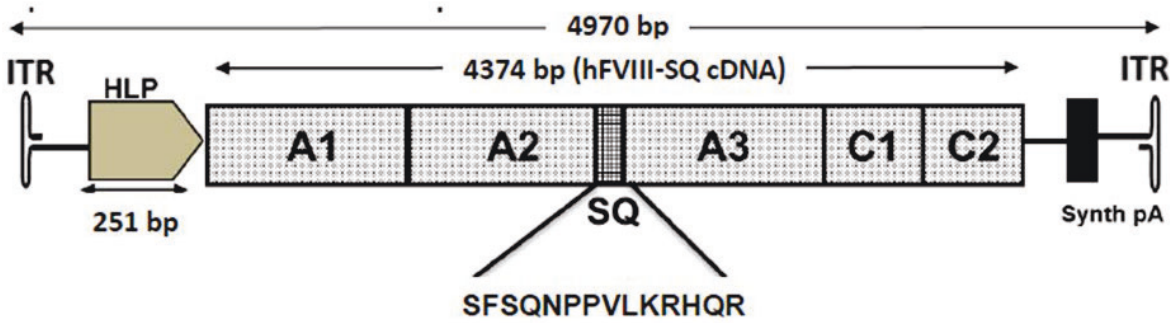
Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011, Haemophilia.). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia.), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci, 2001, N.Engl.J.Med.). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol.Rep.) for serotypes 2, 5 and 8 among others. Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, 2014, N.Engl.J.Med.). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.), (Bainbridge, 2008, N.Engl.J.Med.), (Maguire, 2009, Lancet); (Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.3.1 Liver Biopsy Substudy Rationale

The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms. It is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans.

Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or the variations in response to FVIII gene therapy are

known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown.

The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to genome circularization, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.

7.4 Summary of Overall Risks and Benefits

The majority of subjects in the ongoing 270-201 clinical study who have received 4E13 or 6E13 vg/kg doses of BMN 270 have had Grade 1 asymptomatic elevations in ALT. For most subjects, the elevations have reached only slightly above the upper limit of normal (ULN). Based on the effectiveness of transient oral corticosteroid used to suppress a presumed Class 1 (cytotoxic T-cell) response in prior studies with hepatic transduction with AAV vectors (Mingozzi, 2013), subjects were treated with 7-32 weeks of oral corticosteroids prophylactically or in response to the elevations in ALT to ensure preservation of the transduced hepatocytes. Using this approach, no sustained loss of FVIII activity has been observed in subjects with ALT elevations, consistent with maintaining a high level of hepatocyte function. Moreover, the rise in ALT levels were not accompanied by significant or lasting aberrations in other liver tests such as AST, bilirubin or albumin, indicating that extent of toxicity is limited. There has been one HIV-positive subject in the ongoing 270-302 clinical study who experienced Grade 3 asymptomatic elevations in ALT and AST, which has been attributed to an interaction between one or more of his antiretroviral therapy medications and/or unsuspected underlying hepatic disease with BMN 270. In addition, there has been one subject with Gilbert's syndrome in the ongoing 270-301 clinical study who has experienced Grade 3 asymptomatic elevations in ALT and AST. These cases have led to the exclusion of subsequent HIV-positive subjects and requirement of liver tests at Screening that are <1.25 times the upper limit of the normal range in the ongoing 270-301 and 270-302 clinical studies. Of note, two HIV-positive subjects in 270-301 and one presumed Gilbert's syndrome subject in 270-201 have received BMN 270 without experiencing any elevations in ALT to date. Overall, the literature and clinical experience with BMN 270 suggests that transient elevations in liver enzymes are expected following AAV-based gene therapy for the treatment for hemophilia A or B without any long-term concerns of hepatic injury (Manno, 2006; Nathwani, 2011; George, 2016; Miesbach, 2016; Pasi, 2016).

The current data available for BMN 270 does not yet permit comprehensive assessment of the benefit:risk profile of this investigational drug. Given the monitoring measures in place in the clinical protocol(s) to minimize the risk to subjects participating in the existing studies, the identified risks are justified by the anticipated benefits that may be afforded to subjects. Each subject in 270-201 will have a comprehensive surveillance plan that monitors LTs during the study, and elevations in LT will be addressed according to the guidelines set forth in the protocol. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

For additional information on safety findings in 270-201, refer to current version of the Investigator's Brochure.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

7.4.1 Liver Biopsy Substudy Risks and Benefits

Liver biopsy is considered a safe procedure, with serious complications occurring less than once in every 10,000 procedures (Grant, 2004). Although the theoretical risks of significant complications are extremely small, the main complications would include bleeding and bile leakage. Another theoretical complication is infection at the needle insertion site; the sterile technique used makes this risk extremely small.

The most common problems include mild pain and a minor decrease in blood pressure. More serious complications, such as bleeding, infection, and injury to nearby organs, are very rare, but the subject will be monitored appropriately to ensure correct management should any of these occur. Any complications related to the liver biopsy should be reported as adverse events, as outlined in Section 10. The liver biopsy is a standard investigation, and will be explained more fully by the experienced clinician performing the biopsy.

Each subject who participates in this optional substudy will have a comprehensive pre-/post-biopsy surveillance plan according to the standard procedures at the institution. Safety will be assessed by adverse event reporting and clinical laboratory assessments. Per the Investigator's discretion and/or according to local guidelines, the subject may be kept in overnight following the liver biopsy for additional safety monitoring; such an overnight stay would not be considered a hospitalization for serious adverse event (SAE) reporting purposes (refer to Section 10.4.1.7).

There is no direct benefit from participating in this study other than contributing to understanding the mechanism of action of BMN 270. Consenting into this specific substudy is optional and will not have any effect on the subject’s continued participation in 270-201.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

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 rAAV genomic DNA (eg, concatemers) are present at the time of biopsy
- To determine the transduction pattern in humans (ie, peri-portal hepatocytes, central vein hepatocytes)

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

- Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
- Cohort 2: 2E13 vg per kilogram, iv
- Cohort 3: 6E13 vg per kilogram, iv
- Cohort 4: 4E13 vg per kilogram, iv

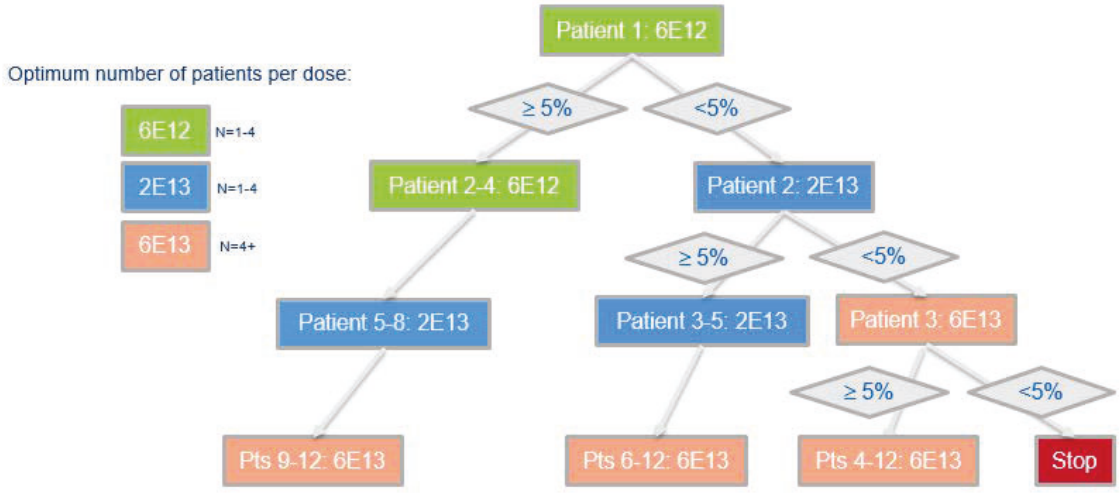
The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in mice.

Cohorts 1-3

The first three cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches ≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme for Cohorts 1 to 3



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the Data Review Board (DRB). The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected PBMC. Additionally, if any of the events listed in Section 9.3.4.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the DRB and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3, Table 9.1.4, and Table 9.1.5 for Safety Follow-up.

Table 9.1.6, dealing with therapeutic corticosteroid use in the event of elevated LTs, is discussed in Section 9.4.8.2.

Liver Biopsy Substudy Design

All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy).

Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL).

Table 9.1.7 presents the schedule of assessments for participation in the liver biopsy substudy.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	Smart Rescreening[i] (Day -28 to Day -1)	Baseline (Day -7 to Day -1)[h]	
Informed consent	X			
Medical History	X			
Physical Examination[a]	X		X	X
Height and Weight[a]	X			
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, coagulation screen, and CRP[e]	X	X	X	
Urine Tests[e]	X	X	X	
Liver Tests[e]	X	X	X	
PBMC collection for CTL baseline			X	
Von Willebrand Factor Antigen (VWF:Ag)	X			
Direct Thrombin Test			X	
PCR of vector DNA in blood, saliva, urine, semen, and stools			X	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

[b] Includes baseline hFVIII activity (chromogenic substrate assay and one-stage clotting assay), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). Sample collection on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion must be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up (Week 1-16)

Assessment	Follow-Up After BMN 270 Administration – Weeks*																
	Week 1																
	D2	D4	D8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical examination[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X		X				X
Urine Tests[b]						X				X				X				X
Liver Tests (local)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer						X				X				X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X	X	X			X		X		X		X		X		X		X
Exploratory biomarker assessments[f]						X				X				X				X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C																		X[g]
PBMC collection			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Von Willebrand Factor Antigen (VWF:Ag)						X				X				X				X
Direct Thrombin test			X												X			

* Visit windows are ± 48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then until at least 3 consecutive negative results are obtained. Collection and testing of semen samples must continue through Week 12, even if 3 consecutive negative samples in that compartment have already been recorded.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[g] Testing for reactivation of hepatitis B and hepatitis C at Week 16, for subjects with a past medical history of Hepatitis B or Hepatitis C prior to study entry, should be performed only in subjects who have not received therapeutic corticosteroids prior to Week 16; subjects who have received therapeutic corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.6.

Table 9.1.3: Schedule of Events – Safety Follow-Up (Week 17-32)

Assessment	Follow-Up After BMN 270 Administration – Weeks*															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight										X						
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X				X				X				X
Urine Tests[b]				X				X				X				X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer				X								X				
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X				X				X				X
Exploratory biomarker assessments[f]				X				X				X				X
Haemo-QoL-A QoL assessment												X				
AAV5 antibody titer				X				X				X				X
PBMC collection	X	X	X	X		X		X		X		X		X		X
Von Willebrand Factor Antigen (VWF:Ag)				X				X				X				X
Direct Thrombin test										X						

* Visit windows are ± 48 hours

[a] Brief physical examination should be done at all weekly visits. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection for each matrix to occur until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.4: Schedule of Events – Safety Follow-Up (Week 33-52)

Assessment	Year 1 – Weeks*											
	33	34	35	36	38	40	42	44	46	48	50	52
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X
Weight[a]												X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X
Urine Tests[b]				X		X		X		X		X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X
AAV5 antibody titer				X		X		X		X		X
FVIII antibody titer				X				X				X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)		X		X				X				X
Von Willebrand Factor Antigen (VWF:Ag)				X		X		X		X		X
Direct Thrombin Test					X							X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X		X		X		X		X
Exploratory biomarker assessments[f]				X		X		X		X		X
Haemo-QoL-A QoL assessment												X

* Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52.

[a] Complete physical examination should be performed at Week 52; brief physical examination may be performed at other study visits. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a

subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.5: Schedule of Events – Safety Follow-Up (Years 2-5)

Assessment	Years 2-5*	Year 2*	Years 3-5*	End of Year Visit				ETV
	Q12W	Q4W[h]	Q6W[h]	Year 2	Year 3	Year 4	Year 5	
				W104	W156	W208	W260	
Physical examination[a]	X[a]			X[a]				X
Weight[a]	X[a]			X[a]				X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X				X
Vital Signs	X			X				X
Blood chemistry, haematology, coagulation screen, and CRP[b]	X			X				X
Urine Tests[b]	X			X				X
Liver Tests (local)[b]	X	X	X	X				X
FVIII assays (local)[c]	X	X	X	X				X
Liver Tests (central)[b]	X	X	X	X				X
FVIII assays (central)[d]	X	X	X	X				X
AAV5 antibody titer	X			X				X
FVIII antibody titer	X			X				X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)	X			X				X
Von Willebrand Factor Antigen (VWF:Ag)	X			X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X[e]	X[e]	X[e]	X[e]				X
Exploratory biomarker assessments[g]	X			X				X
Haemo-QoL-A QoL assessment	X[f]			X[f]				X

* Visit windows are ± 2 weeks for visits in Years 2-5. The Q6W visits during Years 3-5 should restart after each End of Year visit (eg, the first Q6W visit during Year 3 should be ~6 weeks after the End of Year 2 visit).

[a] Complete physical examination should be performed at the End of Year visits; brief physical examination may be performed at other study visits. Weight should be recorded at the second Q12W visit each year and at every End of Year visit during Years 2-5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are ≥1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT ≥ 1.5x ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. During Years 2-5 of the Post-Infusion Follow-Up period, urine tests and blood, chemistry, and coagulation tests should be performed at the second Q12W visit each year and at every End of Year visit.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) during Years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) in years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

[f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at the second Q12W visit each year and at every End of Year visit.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Subjects who meet the definition of treatment failure to BMN 270 therapy after Week 52 may omit the Q4W and Q6W visits during Years 2-5, and must attend only the Q12W and End of Year visits. Such subjects following the abbreviated schedule who have not yet cleared vector shedding in all fluids must still provide samples Q4W (during Year 2) or Q6W (during Years 3-5) until vector shedding has been cleared (but do not need to do other scheduled assessments at those visit dates).

Table 9.1.6: Schedule of Events – Therapeutic Corticosteroids for LT Elevations

	Steroid Treatment Period[d]											Post-Steroid Period[c]				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11[b]**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 13**
Therapeutic corticosteroids (dose in mg/day) [a]	60 mg	60 mg	40 mg	40 mg	30 mg	30 mg	20 mg	20 mg	15 mg	10 mg	5 mg					
FVIII activity testing												X	X	X	X	
Liver testing												X	X	X	X	
Hepatitis B testing[e]						X						X				X
HCV Viral Load[e]						X						X				X

[a] Therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor.

[b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

[c] After discontinuation of corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. If these assessments are already being done as part of normal study follow-up, they do not need to be duplicated.

[d] Regardless of the assessments listed in the Schedule of Assessments (Table 9.1.2, Table 9.1.3, or Table 9.1.4), subjects initiated on corticosteroids will only be required to have laboratory evaluations on a weekly basis.

[e] Should only be performed in subjects with a history of hepatitis B or hepatitis C prior to study entry.

Table 9.1.7: Schedule of Events – Liver Biopsy Substudy

	Within 28 Days Before Biopsy Day	Within 7 Days Before Biopsy Day	1 Day Before Biopsy Day [d]	Biopsy Day (BD)
Informed Consent	X			
Liver Ultrasound[a]	X			
Brief Physical Examination	X		X	X
Hematology, Coagulation, Chemistry Assessments[b]	X		X	
Liver Tests[b]	X		X	X
FibroScan		X		
Local FVIII Activity Level Assessment		X	X	X
Pre-Biopsy Consultation[c]		X		
Liver Biopsy[e]				X

[a] Liver ultrasound must be performed within 28 days prior to the scheduled biopsy, unless an ultrasound result from the prior 3 months is already available.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests.

[c] Subjects will undergo a pre-biopsy consultation with the investigator (treating hematologist).

[d] Subjects may need to repeat/extend this visit if their FVIII activity levels need adjustment before the biopsy, at the discretion of the investigator (treating hematologist).

[e] Biopsy will be a percutaneous or transjugular biopsy under ultrasound guidance, performed according to the standard procedure of the institution. If only a small amount of tissue (< 2 cm) is obtained at the time of the biopsy, the subject may be asked to consent for a second pass. In this case, the original < 2 cm sample should still be retained and handled according to the instructions for handling biopsy specimens in the Laboratory Manual. Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
    - ALT (alanine transaminase) to 3 times the upper limit of normal;
    - Bilirubin above 3 times the upper limit of normal;
    - Alkaline phosphatase above 3 times the upper limit of normal; or
    - INR (international normalized ratio) ≥ 1.4.
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Liver Biopsy Substudy Inclusion and Exclusion Criteria

Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria:

1. Currently enrolled in 270-201
2. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy
3. Able to sign informed consent and comply with requirements of the substudy
4. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy:

1. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to):
   - Significant thrombocytopenia (platelet count < 100 x 10^9/L)
   - Evidence of significant ascites
   - Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.

9.3.4 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- Any ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270, in the absence of a definitive alternate etiology for the increase
- The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion
- The detection of AAV vector DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than 52 weeks after BMN 270 infusion, as discussed in Section 9.7.8.6
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt:

- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days

9.3.5 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.6 Duration of Subject Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical examination performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (eg, the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be discharged from the clinic unless toxicity has been observed in which case the stay in the clinic may be extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

For Cohorts 1 to 3, approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in that cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design for Cohorts 1-3

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents
- Emicizumab
- Fitusiran
- Concizumab
- Efavirenz
- Lamivudine

Subjects who begin antiretroviral therapy following BMN 270 infusion and during their participation in 270-201 must have their antiretroviral medication regimen approved by the Investigator and Medical Monitor prior to starting antiretroviral treatment.

Medications which are predominately metabolized by the liver (eg, acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52). LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

9.4.8.2.1 Therapeutic Corticosteroids

In general, therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator.

Reports of raised LTs (defined as ALT ≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

Following initiation or completion of therapeutic corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

Treatment with prednisolone will be initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.6).

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

Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. For the subjects with a past medical history of hepatitis B or hepatitis C prior to study entry, Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on a dispensing log. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data, if allowed by local SOPs.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials (or must be referenced in their institution SOPs).

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin

and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a study drug return form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

Subjects should be advised to abstain from any blood or sperm donation after BMN 270 infusion, until there is no further evidence of vector shedding from PCR analysis of samples.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.7.1.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1 through Table 9.1.5) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to expression of FVIII at 5 IU/dL or above, (ie, a mild severity). This is associated in natural history studies with clinically superior long term outcomes (Den Ujil, 2011, Haemophilia).

The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic substrate assay)
- FVIII activity by one-stage clotting assay

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

The FVIII activity level in both assays and the number of subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.

FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5 but will be used in conjunction with local lab LT assessments to monitor subject safety and need for initiation of therapeutic corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine

anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage clotting and/or chromogenic substrate assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5.

9.7.6 Exploratory Assessments

Blood samples will be collected at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

Liver biopsy samples collected as part of the exploratory substudy may also be tested to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer. Subjects participating in the substudy may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed.

On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with samples collected specifically for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.7.1 Liver Biopsy Substudy

The objectives of the liver biopsy substudy are considered exploratory.

Subjects who consent to the procedure will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound exam of the liver within 28 days prior to the procedure (to ensure there is no obstruction to the liver that would interfere with the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate any histopathologic findings on the biopsy). Subjects will also undergo a pre-biopsy consultation with the investigator (treating hematologist).

Within 24 hours prior to the biopsy being performed, subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). FVIII activity levels for this purpose should be assessed at the local laboratory within 7 days before the biopsy, and again the day before the biopsy. As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category “Surgery/Procedure”.

The liver biopsy should be performed in the morning, and the biopsy procedure and follow-up care should be done according to the local standard of care. Additional details for handling the biopsy specimens are provided in the Laboratory Manual.

Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion and/or according to local guidelines.

Safety findings arising from the histopathological analysis of the biopsy sample are subject to Adverse Event reporting (Section 10), and such findings should be further assessed and followed-up as clinically appropriate and in order to safeguard the subject's ongoing medical care (this should be done in consultation with a hepatologist and/or other specialist clinicians if required). In the event fibrotic changes being observed on the biopsy sample, additional follow-up Fibroscans may be considered (with the frequency of subsequent scans at the discretion of the Investigator and/or hepatologist).

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	Haematology	Urine Tests	Other
Albumin	Haemoglobin	Appearance	CRP
BUN	Haematocrit	Color	
Calcium	WBC count	pH	**Coagulation Screen including:**
Chloride	RBC count	Specific gravity	APTT
Total cholesterol	Platelet count	Ketones	PT/INR
CO_2	Differential cell count	Protein	TT
CPK	ABO blood typing*	Glucose	
Creatinine		Bilirubin	
Glucose		Nitrite	
Phosphorus		Urobilinogen	
Potassium		Haemoglobin	
Total protein			
Sodium			
Uric Acid			

BUN, blood urea nitrogen; CO_2, carbon dioxide; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.
* ABO blood typing assessment should be completed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study).

9.7.8.3 Liver and Hepatitis Testing

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active hepatitis B or hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing hepatitis B or hepatitis C infection is exclusionary. Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received as part of their medical history assessment at Screening.

Subjects with a history of hepatitis B or hepatitis C infection prior to study entry will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive

therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

A liver ultrasound and LTs at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

Liver tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. At each time point, the following LTs should be assessed:

Table 9.7.8.3.1: Liver Tests

Liver Tests (LTs)			
Alkaline Phosphatase	AST (SGOT)	Total Bilirubin	LDH
ALT (SGPT)	Direct Bilirubin	GGT	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase

Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

9.7.8.4 HIV Testing

HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.

9.7.8.5 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and at the End of Years visits thereafter. A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems.

At other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, dermatologic, musculoskeletal, and gastrointestinal assessments. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

Height will be recorded at Screening only. Weight will be recorded at Screening, at Weeks 26 and 52 during Year 1, and then at the second Q12W visit each year and at every End of Years visit during Years 2-5.

9.7.8.6 Vector Shedding

Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid but not in motile sperm (Manno, 2006, Nat.Med.). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016, Haemophilia).

During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of vector shedding for possible viral transmission. Bodily

fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor). Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule (refer to Section 12.5.3 but who have not cleared vector shedding from all fluids must still provide samples for assessment until vector shedding has cleared: every 4 weeks (during Year 2), or every 6 weeks (during Years 3-5).

Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples (upon consultation between the Investigator and Medical Monitor).

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (eg, sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.1.1.1 Bleeding and Suspected Bleeding Events

All bleeding events and suspected bleeding events, regardless of the need for exogenous FVIII therapy as treatment, should be captured in subject diaries and recorded on the designated bleeding eCRF. Bleeding events and suspected bleeding events should not be reported as adverse events, with the following exception:

- All bleeding events and suspected bleeding events which meet one or more of the criteria for being serious (refer to Section 10.2) should be reported as serious adverse events (whether or not they are bleeding events that are normal sequelae of hemophilia, and whether or not they required exogenous FVIII as treatment).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening
- *Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.*
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (eg, anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of ALT ≥1.5x ULN, regardless of whether that triggers an initiation or modification of corticosteroid treatment
- Thromboembolic event

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.1.1.1.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.1.1.1 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.1.1.1. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE (or SAE, as appropriate) eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. Events that change in severity necessitate the recording of an additional AE. AEs that do not have a change in severity should be recorded only once on the eCRF.

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE or EOSI should be reported as such, and recorded in the AE eCRF. Any laboratory result abnormality of CTCAE Grade 4 or 5 should be recorded as an SAE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Requiring a change in concomitant therapy (eg, addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.1.1.1).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated procedure or assessment
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Insert an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug or FVIII replacement therapy.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record “Unexplained Death” or “Death of Unknown Cause” on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up eCRF. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to

protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.

Address	105 Digital Drive Novato, CA 94949
Phone:	PI
Fax:	PI
E-mail:	drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name:	PI , MA MB BChir MSc
Address:	Biomarin (UK) Ltd. 10 Bloomsbury Way London WC1A 2SL
Phone	PI (office) PI (mobile)
E-mail:	PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic substrate assay and the one-stage clotting assay are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (+ 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, hepatitis B, hepatitis C, and HIV.
    - Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received. Any prior pharmacokinetics information obtained while the subject was receiving prophylactic or on-demand FVIII therapy prior to the study should also be collected. For subjects who have already enrolled in 270-201, this information should be collected at the next regularly scheduled study visit (or at least prior to the subject's completion of study participation).
- Complete Physical Examination
- Height and weight
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic substrate (plasma)

  - Baseline hFVIII activity level – one-stage clotting assay
  - hFVIII coagulation activity exploratory assay
  - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
  - hFVIII total antibody assay
  - hFVIII antigen (ELISA)
- Blood sample for AAV5 Assays
  - AAV5 antibody titer
  - AAV5 transduction inhibition assay
- Screen for hepatitis B, hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Tests (refer to Table 9.7.8.3.1)
- Von Willebrand Factor Antigen (VWF:Ag)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)

- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Tests (refer to Table 9.7.8.3.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Tests (refer to Table 9.7.8.3.1)
- PBMC collection for CTL baseline
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)

- AAV5 antibody titer
- AAV5 transduction inhibition assay

- BMN 270 Infusion
- Vital Signs
  - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Once per week (Weeks 1 through 16)

The following procedures will be performed at one visit per week from Weeks 1 through 16:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
  - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- Samples for FVIII Assays – central laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for

subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

  - Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16

- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
  - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
- PBMC collection

12.5.2 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only) – central laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Liver Tests (refer to Table 9.7.8.3.1) (Day 4 only) – central laboratory assessment
- Liver Tests (refer to Table 9.7.8.3.1) (Day 4 only) – local laboratory assessment
- FVIII activity level (Day 4 only) – local laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)

12.5.3 Week 1 – Day 8

On Day 8, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Direct Thrombin test
- Haemo-QoL-A QoL assessment

12.5.4 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) (not assessed at Week 14)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2)
    - Collection to occur until at least 3 consecutive negative results are obtained. Semen samples should continue to be collected and tested through Week 12, even if 3 consecutive negative results in that compartment have been recorded prior to that timepoint.

12.5.5 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedures will be performed:

- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- FVIII antibody titer
- Exploratory biomarker assessments

12.5.6 Week 16

At Week 16, the following procedures will be performed:

- Test for hepatitis B and hepatitis C reactivation (in subjects with a history of hepatitis B or hepatitis C infection prior to study entry)
    - Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

12.5.7 Weeks 8 and 16

At Weeks 8 and 16, the following procedures will be performed:

- AAV5 antibody titer

12.5.8 Weeks 2, 3, 4, and 16

At Weeks 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.5.9 Week 13

At Week 13, the following procedure will be performed:

- Direct Thrombin test

12.6 Safety Follow-Up – Weeks 17-36

After the Post-Infusion Follow-Up visits are complete, subjects will return to the study site for Safety Follow-Up visits from Weeks 17 through Week 36 (± 48 hours), when the following procedures will be completed:

12.6.1 Once per week (Weeks 17 through 36)

Once per week from Week 17 through Week 36, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

12.6.2 Once per week (Weeks 17 through 20)

Once per week from Week 17 through Week 20, the following procedures will be performed:

- PBMC collection

12.6.3 Every 2 weeks (Weeks 21 through 36)

Every 2 weeks (Weeks 22, 24, 26, 28, 30, 32, 34, and 36), the following procedures will be performed:

- PBMC collection

12.6.4 Every 4 Weeks

Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- AAV5 antibody titer
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.5 Every 8 Weeks

Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed:

- FVIII antibody titer

12.6.6 Week 26

At Week 26, the following procedure will be performed:

- Direct Thrombin test
- Weight

12.6.7 Week 28

At Week 28, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Weeks 37-52

Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:

12.7.1 Once per visit

At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

    Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

12.7.2 Every 4 Weeks

Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.7.3 Every 8 Weeks

Every 8 weeks (Weeks 44 and 52), the following procedure will be performed:

- PBMC collection
- FVIII antibody titer

12.7.4 Week 38 and 52

At Week 38 and Week 52, the following procedure will be performed:

- Direct Thrombin test

12.7.5 Week 52

At Week 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment
- Weight

12.8 Safety Follow-Up – Years 2-5

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52 of the study by attending only the Q12W and End of Year visits during Years 2-5.

Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until vector shedding has cleared (but do not have to perform other scheduled assessments at those study visits).

During Years 2-5 of Safety Follow-up, the following procedures will be completed:

12.8.1 Year 2 – Every 4 Weeks (not required for treatment failure)

During Year 2, every 4 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Year 2 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks during Year 2 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).
    - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks during Year 2 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.2 Years 3-5 – Every 6 Weeks (not required for treatment failure)

During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment

  - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII activity level – local laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
  - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
  - Sample testing during Years 3-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52 and/or Year 2. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 6 weeks during Years 3-5 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).
  - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 6 weeks during Years 3-5 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.3 Years 2-5 – Every 12 Weeks and End of Year Visits (required for all subjects)

During Years 2-5, subjects will be asked to return to the study site for visits at the following study weeks (±2 weeks):

- Year 2 – Week 64, Week 76, Week 88, Week 104
- Year 3 – Week 116, Week 128, Week 140, Week 156
- Year 4 – Week 168, Week 180, Week 192, Week 208
- Year 5 – Week 220, Week 232, Week 244, Week 260

For each of these years, the last study visit listed (Week 104, Week 156, Week 208, and Week 260) will serve as an End of Year visit.

At the every 12 week and End of Year visits, the following procedures will be performed:

- Brief physical examination (at every 12 week visits) or complete physical examination (at End of Year visits)
- Weight (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level

- FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
  - ABO blood typing assessment should be performed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Exploratory biomarker assessments
- Haemo-QoL-A QOL assessment (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
  - Sample testing during Years 2-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.9 Liver Biopsy Substudy - Years 1-5

For the optional liver biopsy substudy (refer to Table 9.1.7), the following procedures will be performed at the following times:

- Within 28 days before Biopsy Day
  - Informed consent
  - Liver ultrasound

        - Liver ultrasound does not need to be performed if an ultrasound result from the prior 3 months is already available
    - Brief physical examination
    - Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.8.2.1)
    - Liver Tests (refer to Table 9.7.8.3.1)
- Within 7 Days before Biopsy Day
    - FibroScan
    - Local laboratory FVIII activity level assessment
    - Pre-biopsy consultation with investigator (treating hematologist). This may need to be repeated/extended if the subject's FVIII activity levels need adjustment before the biopsy, at the discretion of the investigator (treating hematologist).
- 1 Day before Biopsy Day
    - Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.8.2.1)
    - Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - Brief physical examination
    - Local laboratory FVIII activity level assessment
        - Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".
- Biopsy Day
    - Brief physical examination
    - Local laboratory FVIII activity level assessment (performed pre-biopsy)

- Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure".

- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment (pre-biopsy)
- Liver Biopsy (refer to Laboratory Manual for guidelines for biopsy procedure and handling of biopsy samples)
    - If only a small amount of tissue is obtained at the time of the biopsy, the subject may be asked to consent for a second pass.

Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion.

12.10 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 260 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)

    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Exploratory biomarker assessment
- Haemo-QoL-A QOL assessment

12.11 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 260). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay as measured by the central laboratory at 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Liver Biopsy Substudy Analysis

A separate report presenting and discussing analyses of the exploratory objectives for the optional liver biopsy substudy will be prepared.

14.4 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.5 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by a one-stage clotting assay and/or chromogenic substrate assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.6 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.7 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.8 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.9 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas AND to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572 and/or principles of ICH E6 R2 GCP, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and/or ICH E6 R2 Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/ IEC review and approval in 21 CFR Part 56 and/or ICH E6 R2 Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH E6 R2 Section 4.11.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH E6 R2 Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH E6 R2 Section 4.9.7.
- The IRB/IEC/REB complies with the requirements of 21 CFR Part 56, ICH E6 R2 Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201 Amendment 8

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including ICH E6 R2, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name _____

Accepted for the Sponsor: DocuSigned by: PI

Medical Monitor Signature Date

Printed name: PI, MA MB BChir MSc, PI, Clinical Science___

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See page 2). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Revision	Rationale
Synopsis (Study Rationale)	**Liver Biopsy Substudy Rationale** The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms, and it is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans. Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown. The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to circularized genomes, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.	1
Synopsis (Objectives)	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 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)	1

Section No./Title	Revision	Rationale
Synopsis (Study Design and Plan)	**Liver Biopsy Substudy Design** All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy). Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL).	1
Synopsis (Inclusion and Exclusion Criteria)	Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria: 5. Currently enrolled in 270-201 6. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy 7. Able to sign informed consent and comply with requirements of the substudy 8. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator. Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy: 9. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to): • Significant thrombocytopenia (platelet count < 100 x 10^9/L) • Evidence of significant ascites	1

Section No./Title	Revision	Rationale
	• Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.	
Synopsis (Criteria for Evaluation)	Liver Biopsy Substudy: The following exploratory assessments will be performed as part of the optional liver biopsy substudy: • Morphologic/pathogenic changes after FVIII gene transduction or any change that may be associated with sustained ALT rise • Determine quantities of liver FVIII-SQ DNA/RNA • Determine forms of vector DNA in liver at the time of biopsy • Determine percentage of hepatocytes expressing FVIII protein • Determine percentage of hepatocytes staining positive for vector DNA • Determine hepatic liver transcriptome at single nuclei level if sufficient material is obtained • Identify the forms of vector DNA in the liver • Examination of potential stress inducing cellular pathways • Other exploratory assessment to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer	1
7.3.1/Liver Biopsy Substudy Rationale	The pattern of response in hFVIII activity observed so far after administration of BMN 270 demonstrates peak expression levels during the first 6-12 months post-treatment followed by a decline to a steady-state level of expression in the second year of follow-up, with mean hFVIII activity levels remaining above the lower limit of normal (50 IU/dL). One of the explanations may lie in the kinetics of vector genome processing which involves a series of steps such as DNA degradation and repair, annealing, and circularization that can result in the formation of stable double-stranded circularized transgene DNA forms. It is these circularized DNA species that are thought to be associated with long-term, persistent expression of the gene product in target cells. Examination of transduced hepatocytes from subjects treated with BMN 270 in the 270-201 study will help to establish whether DNA circularization may occur and could account for the long-term hFVIII expression observed in humans. Additionally, health of the liver after gene transduction has been monitored indirectly by periodic assessments of hepatic enzymes released into the blood stream. Transient post-treatment elevations in ALT levels have been observed in some subjects, as well as inter-subject variability in post-therapy hFVIII levels. Neither the reasons for, nor the significance of, the ALT elevations or the variations in response to FVIII gene therapy are known. Moreover, the effects of BMN 270 on hepatic tissue structure and function are also currently unknown.	1

Section No./Title	Revision	Rationale
	The purpose of this exploratory substudy is to provide a better understanding of the long-term gene expression related to genome circularization, health of the liver, and variation in hFVIII levels observed after gene therapy with BMN 270. Whilst consenting subjects may not derive any direct benefit themselves by participating in the substudy, the overall findings could aid future patients by helping to characterize the means by which long-term efficacy is achieved and the safety of liver-directed gene therapy.	
7.4.1.1/Liver Biopsy Substudy Risks and Benefits	Liver biopsy is considered a safe procedure, with serious complications occurring less than once in every 10,000 procedures (Grant, 2004). Although the theoretical risks of significant complications are extremely small, the main complications would include bleeding and bile leakage. Another theoretical complication is infection at the needle insertion site; the sterile technique used makes this risk extremely small. The most common problems include mild pain and a minor decrease in blood pressure. More serious complications, such as bleeding, infection, and injury to nearby organs, are very rare, but the subject will be monitored appropriately to ensure correct management should any of these occur. Any complications related to the liver biopsy should be reported as adverse events, as outlined in Section 10. The liver biopsy is a standard investigation, and will be explained more fully by the experienced clinician performing the biopsy. Each subject who participates in this optional substudy will have a comprehensive pre-/post-biopsy surveillance plan according to the standard procedures at the institution. Safety will be assessed by adverse event reporting and clinical laboratory assessments. Per the Investigator's discretion and/or according to local guidelines, the subject may be kept in overnight following the liver biopsy for additional safety monitoring; such an overnight stay would not be considered a hospitalization for serious adverse event (SAE) reporting purposes (refer to Section 10.4.1.7). There is no direct benefit from participating in this study other than contributing to understanding the mechanism of action of BMN 270. Consenting into this specific substudy is optional and will not have any effect on the subject's continued participation in 270-201.	1
8/Study Objectives	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)	1

Section No./Title	Revision	Rationale
	• To quantify FVIII DNA, RNA, and protein expression within hepatocytes • To determine which forms of rAAV genomic DNA (eg, concatemers) are present at the time of biopsy • To determine the transduction pattern in humans (ie, peri-portal hepatocytes, central vein hepatocytes)	
9.1/Overall Study Design and Plan	**Liver Biopsy Substudy Design** All subjects enrolled in 270-201 and who are at least one year post-BMN 270 infusion are eligible for the optional liver biopsy substudy. Subjects who consent to participate in the substudy will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound examination of the liver within 3 months prior to the procedure (to ensure there are no pathological findings such as bile duct obstruction that might interfere with the safe performance of the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate with any potential histopathologic findings of fibrosis on the biopsy). Subjects who consent should have their FVIII levels monitored/adjusted by the Investigator to enable the procedure to be performed safely. This may require the administration of exogenous FVIII replacement products in order to achieve the desired FVIII activity. The target FVIII activity level within 24 hours prior to the liver biopsy is at the discretion of the Investigator and/or according to local guidelines, but at a minimum should be at the lower limit of the normal range (ie, at least 50 IU/dL). Table 9.1.7 presents the schedule of assessments for participation in the liver biopsy substudy.	1
Table 9.1.5/Safety Follow-Up	The Direct Thrombin Test has been removed from this schedule of events and footnote g.	2
Table 9.1.7/Liver Biopsy Substudy	Table 9.1.7 has been added as part of this amendment.	1
9.3.3/Liver Biopsy Substudy Inclusion and Exclusion Criteria	Individuals eligible to participate in the liver biopsy substudy must meet all of the following criteria: 2. Currently enrolled in 270-201 10. Received BMN 270 infusion at least 1 year prior to enrollment in the substudy 11. Able to sign informed consent and comply with requirements of the substudy	1

Section No./Title	Revision	Rationale
	12. FVIII activity at least >50 IU/dL (or higher, depending on local guidelines and/or Investigator discretion) within 24 hours prior to the liver biopsy being performed. (FVIII levels should be assessed at the local laboratory.) Subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII levels to an appropriate level, under the supervision/instruction of the Investigator. Individuals who meet any of the following exclusion criteria will not be eligible to participate in the liver biopsy substudy: 3. Assessed by the investigator or a hepatologist as having a medical condition such that undergoing a liver biopsy would be contraindicated. These conditions could include (but are not limited to): • Significant thrombocytopenia (platelet count < 100 x 10^9/L) • Evidence of significant ascites • Abnormalities detected on liver ultrasound (performed within 90 days of procedure) that would preclude safe performance of the biopsy.	
9.7.6/Exploratory Assessments	Liver biopsy samples collected as part of the exploratory substudy may also be tested to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer. Subjects participating in the substudy may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.	1
9.7.7.1/Liver Biopsy Substudy	The objectives of the liver biopsy substudy are considered exploratory. Subjects who consent to the procedure will have a liver biopsy via either the transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. To enhance safety, all subjects will have an ultrasound exam of the liver within 28 days prior to the procedure (to ensure there is no obstruction to the liver that would interfere with the liver biopsy) and a FibroScan of the liver within one week before the liver biopsy (to correlate any histopathologic findings on the biopsy). Subjects will also undergo a pre-biopsy consultation with the investigator (treating hematologist). Within 24 hours prior to the biopsy being performed, subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). FVIII activity levels for this purpose should be assessed at the local laboratory within 7 days before the biopsy, and again the day before the biopsy. As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under	1

Section No./Title	Revision	Rationale
	the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure". The liver biopsy should be performed in the morning, and the biopsy procedure and follow-up care should be done according to the local standard of care. Additional details for handling the biopsy specimens are provided in the Laboratory Manual. Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion and/or according to local guidelines. Safety findings arising from the histopathological analysis of the biopsy sample are subject to Adverse Event reporting (Section 10), and such findings should be further assessed and followed-up as clinically appropriate and in order to safeguard the subject's ongoing medical care (this should be done in consultation with a hepatologist and/or other specialist clinicians if required). In the event fibrotic changes being observed on the biopsy sample, additional follow-up Fibroscans may be considered (with the frequency of subsequent scans at the discretion of the Investigator and/or hepatologist).	
10.4.1.7/Hospitalization, Prolonged Hospitalization, or Surgery	There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to: • Perform a protocol-mandated ~~efficacy measurement~~ procedure or assessment	1
12.8.3/Years 2-5 – Every 12 Weeks and End of Year Visits	At the every 12 week and End of Year visits, the following procedures will be performed: • ~~Direct Thrombin test~~	2

12.9/Liver Biopsy Substudy – Years 1-5	For the optional liver biopsy substudy (refer to Table 9.1.7), the following procedures will be performed at the following times:
	
	• Within 28 days before Biopsy Day
	  ○ Informed consent
	  ○ Liver ultrasound
	  ▪ Liver ultrasound does not need to be performed if an ultrasound result from the prior 3 months is already available
		1

Section No./Title	Revision	Rationale
	○ Brief physical examination ○ Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.8.2.1) ○ Liver Tests (refer to Table 9.7.8.3.1) • Within 7 Days before Biopsy Day ○ FibroScan ○ Local laboratory FVIII activity level assessment ○ Pre-biopsy consultation with investigator (treating hematologist). This may need to be repeated/extended if the subject's FVIII activity levels need adjustment before the biopsy, at the discretion of the investigator (treating hematologist). • 1 Day before Biopsy Day ○ Hematology, Coagulation, and Chemistry Assessments (refer to Table 9.7.8.2.1) ○ Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment ○ Brief physical examination ○ Local laboratory FVIII activity level assessment ▪ Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure". • Biopsy Day ○ Brief physical examination	

Section No./Title	Revision	Rationale
	○ Local laboratory FVIII activity level assessment (performed pre-biopsy) ▪ Subjects must have a FVIII activity level of at least 50 IU/dL (or higher, depending on local guidelines and/or investigator discretion). As needed, subjects may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the subject during the procedure. This exogenous FVIII usage (if performed) should be recorded in the eCRF FVIII infusion pages under the category "Surgery/Procedure". ○ Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment (pre-biopsy) ○ Liver Biopsy (refer to Laboratory Manual for guidelines for biopsy procedure and handling of biopsy samples) ▪ If only a small amount of tissue is obtained at the time of the biopsy, the subject may be asked to consent for a second pass. Following completion of the biopsy, the subject should remain in the hospital under observation for at least 4-6 hours. Overnight post-procedure observation may be done at the investigator's discretion.	
12.10/Early Termination Visit	At the Early Termination visit, as many of the following assessments as possible should be done: • ~~Direct Thrombin test~~	2
14.3/Liver Biopsy Substudy Analysis	A separate report presenting and discussing analyses of the exploratory objectives for the optional liver biopsy substudy will be prepared.	1
21/References	Grant A, Neuberger J, Day C, Saxseena S. British Society of Gastroenterology Guidelines on the use of Liver Biopsy in Clinical Practice. 2004. Available at: https://www.bsg.org.uk/resource/bsg-guidelines-on-the-use-of-liver-biopsy-in-clinical-practice.html (last visited 1 December 2018).	1

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI , MA MB BChir MSc BioMarin (UK) Ltd. 10 Bloomsbury Way London, WC1A 2SL
Duration of Subject Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015
Date of Amendment 4:	02 September 2016
Date of Amendment 5:	14 February 2017
Date of Amendment 6:	21 December 2017
Date of Amendment 7:	10 October 2018

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 7

Date: 10 October 2018

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 7 to the BMN 270-201 protocol is provided below.

1. Efavirenz, lamivudine, and experimental hemophilia treatments (emicizumab, fitusiran, and concizumab) have been added to the list of prohibited concomitant medications.

Rationale: An HIV-positive subject in Study 270-302 developed markedly elevated transaminase levels after receiving 4E13 vg/kg of BMN 270. The subject was receiving HAART treatment that included efavirenz and lamivudine for his HIV infection, and it is hypothesized that the combination of BMN 270, one or more of his HAART medications, and/or unsuspected underlying hepatic disease may have contributed to the subject's elevated transaminase levels. Experimental hemophilia treatments are prohibited during the study as they could affect the assessment of FVIII levels in 270-201 subjects.

2. The timing of assessment visits during Years 2-5 has been modified.

Rationale: The original timing (which required visits on every 4 week, every 6 week, and every 3 month schedules) created burdens on sites in trying to align visits within allowed visit windows to minimize subject burden. The revised timing (which has substituted visits every 12 weeks and at specified end of year timepoints for the every 3 month visits) has simplified the calendar of visits and should ease site burden.

3. An abbreviated visit schedule has been made available during Years 2-5 for subjects who are considered to have not responded to BMN 270 therapy.

Rationale: Treatment failure—manifesting as either failure to achieve FVIII activity ≥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, enable subjects to follow an abbreviated visit schedule after Week 52 of the study by attending only the Q12W and End of Year visits during Years 2-5.

Subjects who meet the "treatment failure" definition and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until

vector shedding has cleared (but do not need to perform other assessments at those study visits).

4. Additional details have been included concerning information to be collected as part of the medical history.

Rationale: In subjects with a history of hepatitis B or hepatitis C infection, information on the specific treatments received for that infection should be collected. In addition, any previous pharmacokinetic data collected at the time the subject was receiving on-demand or prophylactic FVIII treatment should be collected. This additional history data could help explain a subject's FVIII response (or lack of FVIII response) to BMN 270. As all subjects have already gone through Screening and reported medical history during 270-201, this additional history should be collected at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study).

5. Language concerning when to consider restarting FVIII prophylaxis following BMN 270 infusion has been modified.

Rationale: The decision for reinstitution of FVIII prophylaxis should be based on clinical grounds (eg, the advent of bleeding episodes), in consultation with the Medical Monitor. The need to reinstitute FVIII prophylaxis if the FVIII activity is below a certain level post-BMN 270 has not yet been clinically established but will be informed by results from this study.

6. Language was added to include ABO testing.

Rationale: ABO blood type results will be obtained to potentially correlate with FVIII activity level results in an exploratory manner. This assessment should be completed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study).

7. Clarified that vector shedding assessments, if required, can be performed at the every 6 week visits during Years 3-5.

Rationale: Previously, the protocol called for vector shedding assessments, if required, to be done every 4 weeks after Week 52 until clearance. During Years 3-5, when subjects are being assessed for FVIII and liver function every 6 weeks, an every 4-week schedule for vector shedding would add to subject burden. Obtaining results every 6 weeks is sufficient to monitor vector shedding for subject safety and also aligns these assessments with the FVIII and liver test assessment visits.

8. Assessment of dermatologic and musculoskeletal systems has been added to the brief physical examination.

Rationale: Both organ systems are routinely assessed as part of comprehensive hemophilia care visits.

9. Testing of the exploratory samples for the Direct Thrombin Activity Test has been clarified as optional.

Rationale: While exploratory samples for Direct Thrombin Activity Test will be collected at the time points indicated in the protocol, analysis of these samples will be optional.

10. Subjects will be advised to abstain from blood or sperm donation after BMN 270 infusion until there is no further evidence of vector shedding.

Rationale: Vector shedding in the blood and semen following BMN 270 infusion has been observed, which could serve for vector transmission if subjects were to donate blood or semen following the BMN 270 infusion.

11. In the event of a positive Bethesda assay result during Years 3-5, an additional sample has been added to be collected within 4 weeks of the visit where the positive result was obtained.

Rationale: During Years 3-5, samples are being regularly collected every 6 weeks. However, to align with EMA guidance (which suggests that a confirmatory test on a second sample should be done within a month after a positive Bethesda assay result), language has been added to require the site to conduct an unscheduled visit within 4 weeks after the date when the positive Bethesda result was obtained.

12. Twice weekly evaluation of liver function tests (LFTs) has been added during times when a subject's ALT is ≥ 3x ULN.

Rationale: The decision is based on clinical experience with subject LFT elevations and use of oral corticosteroids in 270-201. More frequent LFT monitoring may be undertaken following review of a subject's data and upon discussion between the investigator and the medical monitor. However, in order to enhance subject safety, the protocol has been modified to mandate twice weekly LFT monitoring when a subject's ALT is ≥ 3x ULN.

13. The identity of the medical monitor has been updated.

14. Minor administrative changes have been made for consistency and clarity.

Specific changes included in this amendment, including the Synopsis, since Amendment 6 (approved 21 December 2017) are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
TITLE OF STUDY: 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		
PROTOCOL NUMBER: 270-201		
STUDY SITES: Approximately 6-10 sites worldwide.		
PHASE OF DEVELOPMENT: Phase 1/2		
STUDY RATIONALE: Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved. Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and		

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maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 1).

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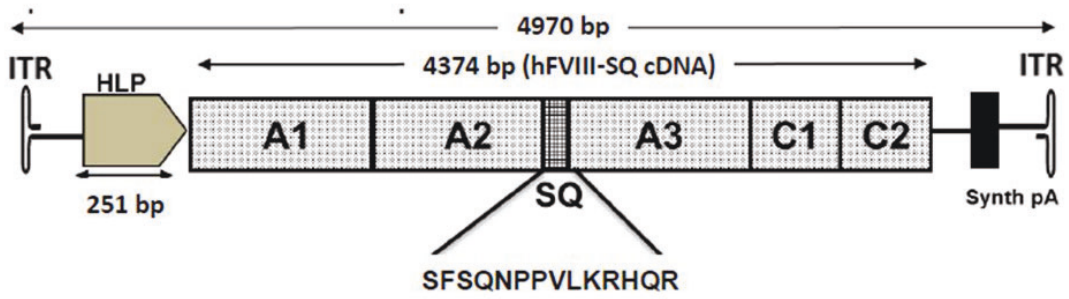
Figure 1. hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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

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STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)

Cohort 2: 2E13 vg per kilogram, iv

Cohort 3: 6E13 vg per kilogram, iv

Cohort 4: 4E13 vg per kilogram, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in non-human primates.

Cohorts 1-3

The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the one-stage clotting and chromogenic substrate assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

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If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator. Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

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NUMBER OF SUBJECTS PLANNED:

Up to 15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;

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- Alkaline phosphatase above 3 times the upper limit of normal; or
- INR (international normalized ratio) ≥ 1.4

5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of < 100 x 10^9/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:
Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:
The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:
BMN 270 is given as a single dose by intravenous infusion.

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CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver tests (LTs, including ALT, AST, GGT, LDH, bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52) during Year 1. LTs will be monitored every 4 weeks during Year 2, and then every 6 weeks for Years 3-5 post-dose in the safety extension; the frequency and duration of LT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage clotting and/or chromogenic substrate assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

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STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay at 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as vector shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
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
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee

IND	Investigational New Drug (application)
INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LT	liver test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver tests (LTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman, 2013, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay, 2012, Blood).

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2013, Haemophilia.); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This

nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in

the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani, 2006, Blood). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Engl.J.Med.), (Nathwani, 2014, N.Engl.J.Med.). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola, 2014, J.Hepatol.). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Mannucci, 2001, N.Engl.J.Med.). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

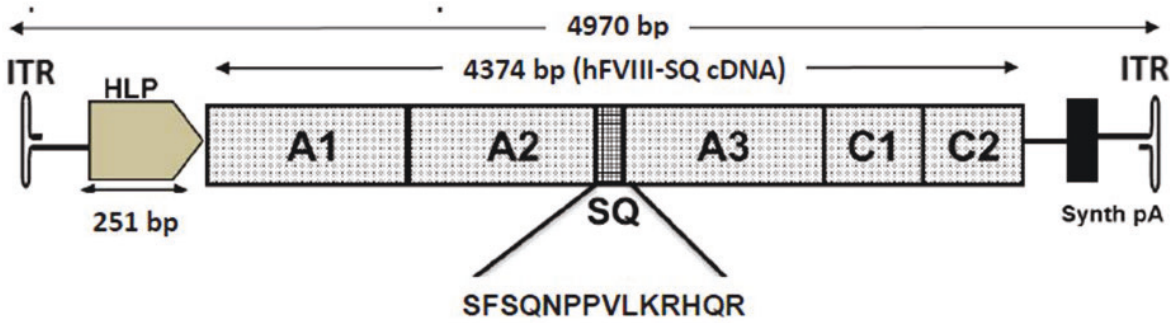
Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011, Haemophilia.). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (eg, direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (eg, FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia.), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci, 2001, N.Engl.J.Med.). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol.Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, 2014, N.Engl.J.Med.). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.), (Bainbridge, 2008, N.Engl.J.Med.), (Maguire, 2009, Lancet); (Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

The majority of subjects in the ongoing 270-201 clinical study who have received 4E13 or 6E13 vg/kg doses of BMN 270 have had Grade 1 asymptomatic elevations in ALT. For most subjects, the elevations have reached only slightly above the upper limit of normal (ULN). Based on the effectiveness of transient oral corticosteroid used to suppress a presumed Class 1 (cytotoxic T-cell) response in prior studies with hepatic transduction with AAV vectors (Mingozzi, 2013), subjects were treated with 7-32 weeks of oral corticosteroids prophylactically or in response to the elevations in ALT to ensure preservation of the transduced hepatocytes. Using this approach, no sustained loss of FVIII activity has been observed in subjects with ALT elevations, consistent with maintaining a high level of hepatocyte function. Moreover, the rise in ALT levels were not accompanied by significant or lasting aberrations in other liver tests such as AST, bilirubin or albumin, indicating that extent of toxicity is limited. There has been one HIV-positive subject in the ongoing 270-302 clinical study who experienced Grade 3 asymptomatic elevations in ALT and AST, which has been attributed to an interaction between one or more of his antiretroviral therapy medications and/or unsuspected underlying hepatic disease with BMN 270. In addition, there has been one subject with Gilbert's syndrome in the ongoing 270-301 clinical study who has experienced Grade 3 asymptomatic elevations in ALT and AST. These cases have led to the exclusion of subsequent HIV-positive subjects and requirement of liver tests at Screening

that are <1.25 times the upper limit of the normal range in the ongoing 270-301 and 270-302 clinical studies. Of note, two HIV-positive subjects in 270-301 and one presumed Gilbert's syndrome subject in 270-201 have received BMN 270 without experiencing any elevations in ALT to date. Overall, the literature and clinical experience with BMN 270 suggests that transient elevations in liver enzymes are expected following AAV-based gene therapy for the treatment for hemophilia A or B without any long-term concerns of hepatic injury (Manno, 2006; Nathwani, 2011; George, 2016; Miesbach, 2016; Pasi, 2016).

The current data available for BMN 270 does not yet permit comprehensive assessment of the benefit:risk profile of this investigational drug. Given the monitoring measures in place in the clinical protocol(s) to minimize the risk to subjects participating in the existing studies, the identified risks are justified by the anticipated benefits that may be afforded to subjects. Each subject in 270-201 will have a comprehensive surveillance plan that monitors LTs during the study, and elevations in LT will be addressed according to the guidelines set forth in the protocol. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

For additional information on safety findings in 270-201, refer to current version of the Investigator's Brochure.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

- Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
- Cohort 2: 2E13 vg per kilogram, iv
- Cohort 3: 6E13 vg per kilogram, iv
- Cohort 4: 4E13 vg per kilogram, iv

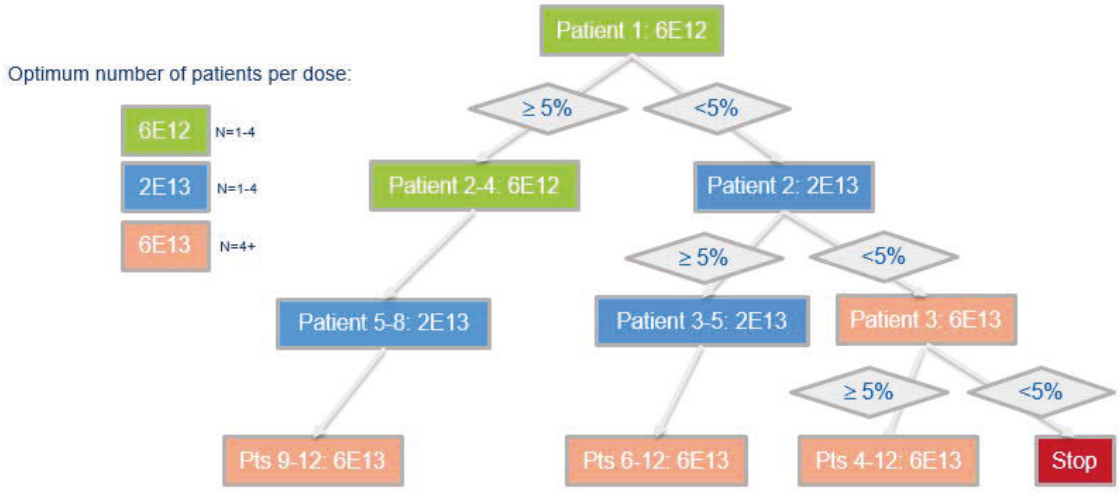
The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in mice.

Cohorts 1-3

The first three cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches ≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme for Cohorts 1 to 3



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the Data Review Board (DRB). The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected PBMC. Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the DRB and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 and Table 9.1.4 for Safety Follow-up.

Table 9.1.6, dealing with therapeutic corticosteroid use in the event of elevated LTs, is discussed in Section 9.4.8.2.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	Smart Rescreening[i] (Day -28 to Day -1)	Baseline (Day -7 to Day -1)[h]	
Informed consent	X			
Medical History	X			
Physical Examination[a]	X		X	X
Height and Weight[a]	X			
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, coagulation screen, and CRP[e]	X	X	X	
Urine Tests[e]	X	X	X	
Liver Tests[e]	X	X	X	
PBMC collection for CTL baseline			X	
Von Willebrand Factor Antigen (VWF:Ag)	X			
Direct Thrombin Test			X	
PCR of vector DNA in blood, saliva, urine, semen, and stools			X	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

[b] Includes baseline hFVIII activity (chromogenic substrate assay and one-stage clotting assay), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). Sample collection on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion must be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up (Week 1-16)

Assessment	Follow-Up After BMN 270 Administration – Weeks*																
	Week 1																
	D2	D4	D8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical examination[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X		X				X
Urine Tests[b]						X				X				X				X
Liver Tests (local)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer						X				X				X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X	X	X			X		X		X		X		X		X		X
Exploratory biomarker assessments[f]						X				X				X				X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C																		X[g]
PBMC collection			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Von Willebrand Factor Antigen (VWF:Ag)						X				X				X				X
Direct Thrombin test			X												X			

* Visit windows are ± 48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting assay and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then until at least 3 consecutive negative results are obtained. Collection and testing of semen samples must continue through Week 12, even if 3 consecutive negative samples in that compartment have already been recorded.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[g] Testing for reactivation of hepatitis B and hepatitis C at Week 16, for subjects with a past medical history of Hepatitis B or Hepatitis C prior to study entry, should be performed only in subjects who have not received therapeutic corticosteroids prior to Week 16; subjects who have received therapeutic corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.6.

Table 9.1.3: Schedule of Events – Safety Follow-Up (Week 17-32)

Assessment	Follow-Up After BMN 270 Administration – Weeks*															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight										X						
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X				X				X				X
Urine Tests[b]				X				X				X				X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer				X								X				
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X				X				X				X
Exploratory biomarker assessments[f]				X				X				X				X
Haemo-QoL-A QoL assessment												X				
AAV5 antibody titer				X				X				X				X
PBMC collection	X	X	X	X		X		X		X		X		X		X
Von Willebrand Factor Antigen (VWF:Ag)				X				X				X				X
Direct Thrombin test										X						

* Visit windows are ± 48 hours

[a] Brief physical examination should be done at all weekly visits. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection for each matrix to occur until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.4: Schedule of Events – Safety Follow-Up (Week 33-52)

Assessment	Year 1 – Weeks*											
	33	**34**	**35**	**36**	**38**	**40**	**42**	**44**	**46**	**48**	**50**	**52**
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X
Weight[a]												X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X
Urine Tests[b]				X		X		X		X		X
Liver Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X
Liver Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X
AAV5 antibody titer				X		X		X		X		X
FVIII antibody titer				X				X				X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)		X		X				X				X
Von Willebrand Factor Antigen (VWF:Ag)				X		X		X		X		X
Direct Thrombin Test					X							X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X		X		X		X		X
Exploratory biomarker assessments[f]				X		X		X		X		X
Haemo-QoL-A QoL assessment												X

* Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52.

[a] Complete physical examination should be performed at Week 52; brief physical examination may be performed at other study visits. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a

subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

Table 9.1.5: Schedule of Events – Safety Follow-Up (Years 2-5)

Assessment	Years 2-5*	Year 2*	Years 3-5*	End of Year Visit				ETV
	Q12W	Q4W[h]	Q6W[h]	Year 2	Year 3	Year 4	Year 5	
				W104	W156	W208	W260	
Physical examination[a]	X[a]			X[a]				X
Weight[a]	X[a]			X[a]				X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X				X
Vital Signs	X			X				X
Blood chemistry, haematology, coagulation screen, and CRP[b]	X			X				X
Urine Tests[b]	X			X				X
Liver Tests (local)[b]	X	X	X	X				X
FVIII assays (local)[c]	X	X	X	X				X
Liver Tests (central)[b]	X	X	X	X				X
FVIII assays (central)[d]	X	X	X	X				X
AAV5 antibody titer	X			X				X
FVIII antibody titer	X			X				X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)	X			X				X
Von Willebrand Factor Antigen (VWF:Ag)	X			X				X
Direct Thrombin Test	X			X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X[e]	X[e]	X[e]	X[e]				X
Exploratory biomarker assessments[g]	X			X				X
Haemo-QoL-A QoL assessment	X[f]			X[f]				X

* Visit windows are ± 2 weeks for visits in Years 2-5. The Q6W visits during Years 3-5 should restart after each End of Year visit (eg, the first Q6W visit during Year 3 should be ~6 weeks after the End of Year 2 visit).

[a] Complete physical examination should be performed at the End of Year visits; brief physical examination may be performed at other study visits. Weight should be recorded at the second Q12W visit each year and at every End of Year visit during Years 2-5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver tests. LTs may be monitored more or less frequently (and in particular when ALT values are ≥ 1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT ≥ 1.5x ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN or increased by > 10 U/L from prior assessment; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. During Years 2-5 of the Post-Infusion Follow-Up period, urine tests and blood, chemistry, and coagulation tests should be performed at the second Q12W visit each year and at every End of Year visit.

[c] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) during Years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[d] Includes hFVIII activity level (one-stage clotting and chromogenic substrate assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) in years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

[f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at the second Q12W visit each year and at every End of Year visit.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.

[h] Subjects who meet the definition of treatment failure to BMN 270 therapy after Week 52 may omit the Q4W and Q6W visits during Years 2-5, and must attend only the Q12W and End of Year visits. Such subjects following the abbreviated schedule who have not yet cleared vector shedding in all fluids must still provide samples Q4W (during Year 2) or Q6W (during Years 3-5) until vector shedding has been cleared (but do not need to do other scheduled assessments at those visit dates).

Table 9.1.6: Schedule of Events – Therapeutic Corticosteroids for LT Elevations

	Steroid Treatment Period[d]											Post-Steroid Period[c]				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11[b]**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 13**
Therapeutic corticosteroids (dose in mg/day) [a]	60 mg	60 mg	40 mg	40 mg	30 mg	30 mg	20 mg	20 mg	15 mg	10 mg	5 mg					
FVIII activity testing												X	X	X	X	
Liver testing												X	X	X	X	
Hepatitis B testing[e]						X						X				X
HCV Viral Load[e]						X						X				X

[a] Therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor.

[b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

[c] After discontinuation of corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. If these assessments are already being done as part of normal study follow-up, they do not need to be duplicated.

[d] Regardless of the assessments listed in the Schedule of Assessments (Table 9.1.2, Table 9.1.3, or Table 9.1.4), subjects initiated on corticosteroids will only be required to have laboratory evaluations on a weekly basis.

[e] Should only be performed in subjects with a history of hepatitis B or hepatitis C prior to study entry.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
5. ALT (alanine transaminase) to 3 times the upper limit of normal;
6. Bilirubin above 3 times the upper limit of normal;
7. Alkaline phosphatase above 3 times the upper limit of normal; or
8. INR (international normalized ratio) ≥ 1.4.
9. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
10. Evidence of any bleeding disorder not related to Haemophilia A
11. Platelet count of $< 100 \times 10^9$/L
12. Creatinine ≥ 1.5 mg/dL
13. Liver cirrhosis of any etiology as assessed by liver ultrasound
14. Hepatitis B if surface antigen is positive
15. Hepatitis C if RNA is positive
16. Treatment with any IP within 30 days prior to the end of the screening period
17. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
18. Prior treatment with any gene transfer agent
19. Major surgery planned in the 16-week period following the viral infusion
20. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- Any ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270, in the absence of a definitive alternate etiology for the increase
- The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion
- The detection of AAV vector DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than 52 weeks after BMN 270 infusion, as discussed in Section 9.7.8.6
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt:

- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days

9.3.4 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.5 Duration of Subject Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical examination performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (eg, the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be discharged from

the clinic unless toxicity has been observed in which case the stay in the clinic may be extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

For Cohorts 1 to 3, approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1)

at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in that cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design for Cohorts 1-3

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents
- Emicizumab
- Fitusiran
- Concizumab
- Efavirenz
- Lamivudine

Subjects who begin antiretroviral therapy following BMN 270 infusion and during their participation in 270-201 must have their antiretroviral medication regimen approved by the Investigator and Medical Monitor prior to starting antiretroviral treatment.

Medications which are predominately metabolized by the liver (eg, acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52). LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

9.4.8.2.1 Therapeutic Corticosteroids

In general, therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator.

Reports of raised LTs (defined as ALT ≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available. Local laboratory results of LTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

Following initiation or completion of therapeutic corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

Treatment with prednisolone will be initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.6).

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

Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. For the subjects with a past medical history of hepatitis B or hepatitis C prior to study entry, Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on a dispensing log. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data, if allowed by local SOPs.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials (or must be referenced in their institution SOPs).

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a study drug return form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

Subjects should be advised to abstain from any blood or sperm donation after BMN 270 infusion, until there is no further evidence of vector shedding from PCR analysis of samples.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1 through Table 9.1.5) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of

BMN 270 required to convert severe HA patients to expression of FVIII at 5 IU/dL or above, (ie, a mild severity). This is associated in natural history studies with clinically superior long term outcomes (Den Ujil, 2011, Haemophilia).

The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic substrate assay)
- FVIII activity by one-stage clotting assay

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

The FVIII activity level in both assays and the number of subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.

FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4, but will be used in conjunction with local lab LT assessments to monitor subject safety and need for initiation of therapeutic corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage clotting and/or chromogenic substrate assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.6 Exploratory Assessments

Blood samples will be collected at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed.

On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with samples collected specifically for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	Haematology	Urine Tests	Other
Albumin	Haemoglobin	Appearance	CRP
BUN	Haematocrit	Color	
Calcium	WBC count	pH	**Coagulation Screen including:**
Chloride	RBC count	Specific gravity	APTT
Total cholesterol	Platelet count	Ketones	PT/INR
CO_2	Differential cell count	Protein	TT
CPK	ABO blood typing*	Glucose	
Creatinine		Bilirubin	
Glucose		Nitrite	
Phosphorus		Urobilinogen	
Potassium		Haemoglobin	
Total protein			
Sodium			
Uric Acid			

BUN, blood urea nitrogen; CO_2, carbon dioxide; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.
* ABO blood typing assessment should be completed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study).

9.7.8.3 Liver and Hepatitis Testing

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active hepatitis B or hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing hepatitis B or hepatitis C infection is exclusionary. Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received as part of their medical history assessment at Screening.

Subjects with a history of hepatitis B or hepatitis C infection prior to study entry will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

A liver ultrasound and LTs at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopoenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

Liver tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. At each time point, the following LTs should be assessed:

Table 9.7.8.3.1: Liver Tests

Liver Tests (LTs)			
Alkaline Phosphatase	AST (SGOT)	Total Bilirubin	LDH
ALT (SGPT)	Direct Bilirubin	GGT	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase

Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

9.7.8.4 HIV Testing

HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.

9.7.8.5 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every

15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and at the End of Years visits thereafter. A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems.

At other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, dermatologic, musculoskeletal, and gastrointestinal assessments. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

Height will be recorded at Screening only. Weight will be recorded at Screening, at Weeks 26 and 52 during Year 1, and then at the second Q12W visit each year and at every End of Years visit during Years 2-5.

9.7.8.6 Vector Shedding

Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid but not in motile sperm (Manno, 2006, Nat.Med.). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016, Haemophilia).

During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of vector shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, Table 9.1.4, and Table 9.1.5. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine

- Stool

Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor). Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule (refer to Section 12.5.3 but who have not cleared vector shedding from all fluids must still provide samples for assessment until vector shedding has cleared: every 4 weeks (during Year 2), or every 6 weeks (during Years 3-5).

Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples (upon consultation between the Investigator and Medical Monitor).

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (eg, sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.1.1.1 Bleeding and Suspected Bleeding Events

All bleeding events and suspected bleeding events, regardless of the need for exogenous FVIII therapy as treatment, should be captured in subject diaries and recorded on the designated bleeding eCRF. Bleeding events and suspected bleeding events should not be reported as adverse events, with the following exception:

- All bleeding events and suspected bleeding events which meet one or more of the criteria for being serious (refer to Section 10.2) should be reported as serious adverse events (whether or not they are bleeding events that are normal sequelae of hemophilia, and whether or not they required exogenous FVIII as treatment).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening
- *Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.*
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (eg, anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of ALT ≥1.5x ULN, regardless of whether that triggers an initiation or modification of corticosteroid treatment
- Thromboembolic event

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.1.1.1.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.1.1.1 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.1.1.1. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE (or SAE, as appropriate) eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. Events that change in severity necessitate the recording of an additional AE. AEs that do not have a change in severity should be recorded only once on the eCRF.

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE or EOSI should be reported as such, and recorded in the AE eCRF. Any laboratory result abnormality of CTCAE Grade 4 or 5 should be recorded as an SAE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Requiring a change in concomitant therapy (eg, addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.1.1.1).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Insert an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug or FVIII replacement therapy.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record “Unexplained Death” or “Death of Unknown Cause” on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up eCRF. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to

protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.

Address	105 Digital Drive Novato, CA 94949
Phone:	PI
Fax:	PI
E-mail:	drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name:	PI, MA MB BChir MSc
Address:	Biomarin (UK) Ltd. 10 Bloomsbury Way London WC1A 2SL
Phone	PI (office) PI (mobile)
E-mail:	PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic substrate assay and the one-stage clotting assay are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, hepatitis B, hepatitis C, and HIV. Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received. Any prior pharmacokinetics information obtained while the subject was receiving prophylactic or on-demand FVIII therapy prior to the study should also be collected. For subjects who have already enrolled in 270-201, this information should be collected at the next regularly scheduled study visit (or at least prior to the subject's completion of study participation).
- Complete Physical Examination
- Height and weight
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic substrate (plasma)
    - Baseline hFVIII activity level – one-stage clotting assay
    - hFVIII coagulation activity exploratory assay

  - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
  - hFVIII total antibody assay
  - hFVIII antigen (ELISA)
- Blood sample for AAV5 Assays
  - AAV5 antibody titer
  - AAV5 transduction inhibition assay
- Screen for hepatitis B, hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Tests (refer to Table 9.7.8.3.1)
- Von Willebrand Factor Antigen (VWF:Ag)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- Blood sample for AAV5 Assays
  - AAV5 antibody titer

- AAV5 transduction inhibition assay

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Tests (refer to Table 9.7.8.3.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Tests (refer to Table 9.7.8.3.1)
- PBMC collection for CTL baseline
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay

- BMN 270 Infusion
- Vital Signs
    - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Once per week (Weeks 1 through 16)

The following procedures will be performed at one visit per week from Weeks 1 through 16:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- Samples for FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16

- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
  - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
- PBMC collection

12.5.2 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only) – central laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Liver Tests (refer to Table 9.7.8.3.1) (Day 4 only) – central laboratory assessment
- Liver Tests (refer to Table 9.7.8.3.1) (Day 4 only) – local laboratory assessment
- FVIII activity level (Day 4 only) – local laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)

12.5.3 Week 1 – Day 8

On Day 8, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Direct Thrombin test
- Haemo-QoL-A QoL assessment

12.5.4 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) (not assessed at Week 14)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2)
    - Collection to occur until at least 3 consecutive negative results are obtained. Semen samples should continue to be collected and tested through Week 12, even if 3 consecutive negative results in that compartment have been recorded prior to that timepoint.

12.5.5 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedures will be performed:

- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- FVIII antibody titer
- Exploratory biomarker assessments

12.5.6 Week 16

At Week 16, the following procedures will be performed:

- Test for hepatitis B and hepatitis C reactivation (in subjects with a history of hepatitis B or hepatitis C infection prior to study entry)
    - Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.6.

12.5.7 Weeks 8 and 16

At Weeks 8 and 16, the following procedures will be performed:

- AAV5 antibody titer

12.5.8 Weeks 2, 3, 4, and 16

At Weeks 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.5.9 Week 13

At Week 13, the following procedure will be performed:

- Direct Thrombin test

12.6 Safety Follow-Up – Weeks 17-36

After the Post-Infusion Follow-Up visits are complete, subjects will return to the study site for Safety Follow-Up visits from Weeks 17 through Week 36 (± 48 hours), when the following procedures will be completed:

12.6.1 Once per week (Weeks 17 through 36)

Once per week from Week 17 through Week 36, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

12.6.2 Once per week (Weeks 17 through 20)

Once per week from Week 17 through Week 20, the following procedures will be performed:

- PBMC collection

12.6.3 Every 2 weeks (Weeks 21 through 36)

Every 2 weeks (Weeks 22, 24, 26, 28, 30, 32, 34, and 36), the following procedures will be performed:

- PBMC collection

12.6.4 Every 4 Weeks

Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- AAV5 antibody titer
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.5 Every 8 Weeks

Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed:

- FVIII antibody titer

12.6.6 Week 26

At Week 26, the following procedure will be performed:

- Direct Thrombin test
- Weight

12.6.7 Week 28

At Week 28, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Weeks 37-52

Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:

12.7.1 Once per visit

At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level

- FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

12.7.2 Every 4 Weeks

Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
  - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.7.3 Every 8 Weeks

Every 8 weeks (Weeks 44 and 52), the following procedure will be performed:

- PBMC collection
- FVIII antibody titer

12.7.4 Week 38 and 52

At Week 38 and Week 52, the following procedure will be performed:

- Direct Thrombin test

12.7.5 Week 52

At Week 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

- Weight

12.8 Safety Follow-Up – Years 2-5

Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52 of the study by attending only the Q12W and End of Year visits during Years 2-5.

Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until vector shedding has cleared (but do not have to perform other scheduled assessments at those study visits).

During Years 2-5 of Safety Follow-up, the following procedures will be completed:

12.8.1 Year 2 – Every 4 Weeks (not required for treatment failure)

During Year 2, every 4 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)

- FVIII coagulation activity exploratory assay
- Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
- FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
  - Sample testing during Year 2 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks during Year 2 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).
  - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks during Year 2 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.2 Years 3-5 – Every 6 Weeks (not required for treatment failure)

During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed:

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
  - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment

- LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII Assays – central laboratory assessment
  - FVIII activity level (one-stage clotting assay)
  - FVIII activity level (chromogenic substrate assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
  - Sample testing during Years 3-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52 and/or Year 2. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 6 weeks during Years 3-5 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).
  - Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 6 weeks during Years 3-5 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).

12.8.3 Years 2-5 – Every 12 Weeks and End of Year Visits (required for all subjects)

During Years 2-5, subjects will be asked to return to the study site for visits at the following study weeks (±2 weeks):

- Year 2 – Week 64, Week 76, Week 88, Week 104
- Year 3 – Week 116, Week 128, Week 140, Week 156
- Year 4 – Week 168, Week 180, Week 192, Week 208

- Year 5 – Week 220, Week 232, Week 244, Week 260

For each of these years, the last study visit listed (Week 104, Week 156, Week 208, and Week 260) will serve as an End of Year visit.

At the every 12 week and End of Year visits, the following procedures will be performed:

- Brief physical examination (at every 12 week visits) or complete physical examination (at End of Year visits)
- Weight (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Vital Signs

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
    - ABO blood typing assessment should be performed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test
- Exploratory biomarker assessments
- Haemo-QoL-A QOL assessment (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)
    - Sample testing during Years 2-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.9 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 260 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)

- Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
- Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage clotting assay)
    - FVIII activity level (chromogenic substrate assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Exploratory biomarker assessment
- Haemo-QoL-A QOL assessment

12.10 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 260). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or

noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

14.2 Missing data will not be imputed.

14.3 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic substrate assay and/or one-stage clotting assay as measured by the central laboratory at 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.4 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.5 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage clotting assay and/or chromogenic substrate assay (activity level), will be used for

plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.6 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.7 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.8 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.9 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon

completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572 and/or principles of ICH E6 R2 GCP, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and/or ICH E6 R2 Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/ IEC review and approval in 21 CFR Part 56 and/or ICH E6 R2 Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH E6 R2 Section 4.11.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH E6 R2 Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH E6 R2 Section 4.9.7.
- The IRB/IEC/REB complies with the requirements of 21 CFR Part 56, ICH E6 R2 Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201, Amendment 7

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including ICH E6 R2, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name _____

Accepted for the Sponsor:

DocuSigned by:
PI

Medical Monitor Signature Date

Printed name: PI , MA MB BChir MSc, PI , Clinical Science___

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See pages 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Revision	Rationale
Synopsis (Study Design and Plan)	As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage. In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator. Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).	5, 14
7.4/Summary of Overall Risks and Benefits	~~Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan . Each subject will have a comprehensive surveillance plan that monitors LFTs during the study.~~ The majority of subjects in the ongoing 270-201 clinical study who have received 4E13 or 6E13 vg/kg doses of BMN 270 have had Grade 1 asymptomatic elevations in ALT. For most subjects, the elevations have reached only slightly above the upper limit of normal (ULN). Based on the effectiveness of transient oral corticosteroid used to suppress a presumed Class 1 (cytotoxic T-cell) response in prior studies with hepatic transduction with AAV vectors (Mingozzi, 2013), subjects were treated with 7-32 weeks of oral corticosteroids prophylactically or in response to the elevations in ALT to ensure preservation of the transduced hepatocytes. Using this approach, no sustained loss of FVIII activity has been observed in subjects with ALT elevations, consistent with maintaining a high level of hepatocyte function. Moreover, the rise in ALT levels were not accompanied by significant or lasting aberrations in other liver tests such as AST, bilirubin or albumin, indicating that extent	14

Section No./Title	Revision	Rationale
	of toxicity is limited. There has been one HIV-positive subject in the ongoing 270-302 clinical study who experienced Grade 3 asymptomatic elevations in ALT and AST, which has been attributed to an interaction between one or more of his antiretroviral therapy medications and/or unsuspected underlying hepatic disease with BMN 270. In addition, there has been one subject with Gilbert's syndrome in the ongoing 270-301 clinical study who has experienced Grade 3 asymptomatic elevations in ALT and AST. These cases have led to the exclusion of subsequent HIV-positive subjects and requirement of liver tests at Screening that are <1.25 times the upper limit of the normal range in the ongoing 270-301 and 270-302 clinical studies. Of note, two HIV-positive subjects in 270-301 and one presumed Gilbert's syndrome subject in 270-201 have received BMN 270 without experiencing any elevations in ALT to date. Overall, the literature and clinical experience with BMN 270 suggests that transient elevations in liver enzymes are expected following AAV-based gene therapy for the treatment for hemophilia A or B without any long-term concerns of hepatic injury (Manno, 2006; Nathwani, 2011; George, 2016; Miesbach, 2016; Pasi, 2017). The current data available for BMN 270 does not yet permit comprehensive assessment of the benefit:risk profile of this investigational drug. Given the monitoring measures in place in the clinical protocol(s) to minimize the risk to subjects participating in the existing studies, the identified risks are justified by the anticipated benefits that may be afforded to subjects. Each subject in 270-201 will have a comprehensive surveillance plan that monitors LTs during the study, and elevations in LT will be addressed according to the guidelines set forth in the protocol. Safety will be assessed by adverse event reporting and clinical laboratory assessments.	
9.1/Overall Study Design and Plan	As the relationship between activity assay results of the BMN 270 gene product and bleeding remains to be established, Investigators should strive to minimize bias by avoiding consideration of FVIII activity levels by themselves or subjects in the reporting of bleeding episodes and FVIII usage. In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis. Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.	5, 14

Section No./Title	Revision	Rationale
Table 9.1.1, Table 9.1.2, Table 9.1.3	*Table 9.1.1, Table 9.1.2, and Table 9.1.3 have been updated to reflect the changes made elsewhere in the protocol.*	4, 6, 9, 12, 14
Table 9.1.1 (Footnotes)	[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.	9
Table 9.1.2 (Footnotes)	[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver ~~function~~ tests. ~~LFTs~~LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data~~.~~, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (~~e.g.,~~eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. [f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.	9, 12, 14
Table 9.1.3 (Footnotes)	[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver ~~function~~ tests. ~~LFTs~~LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data~~.~~, but LTs will be monitored at least twice weekly during periods when a subject's ALT is ≥ 3x ULN. Patients with ALT > 1.5 ULN during the study may undergo additional	9, 12, 14

Section No./Title	Revision	Rationale
	evaluations (~~e.g.,~~eg, imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator. Additional testing of FVIII and LTs during any study week may be performed if: (1) the ALT has increased to above ULN; (2) Increases in ALT values from prior assessment are accompanied by declines in FVIII activity level; or after a discussion between the Medical Monitor and the Investigator based on a review of subject data. If FVIII levels and/or ALT values are stable over the course of several weeks, assessment of FVIII activity and ALT levels may be adjusted based on discussion between the Medical Monitor and the Investigator. [e] Collection for each matrix to occur until at least 3 consecutive negative results are obtained. [f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples (including those for Direct Thrombin Activity test and any other exploratory assessments) will be performed only as deemed necessary by the Sponsor.	
Table 9.1.4 and Table 9.1.5	*Table 9.1.4 and Table 9.1.5 and their associated footnotes have been modified/added to reflect changes made elsewhere in the protocol.*	2, 3, 6, 9, 11, 12, 14
9.4.8/Prior and Concomitant Medications	The following medications are prohibited starting 30 days before Screening: • Emicizumab • Fitusiran • Concizumab • Efavirenz • Lamivudine Subjects who begin antiretroviral therapy following BMN 270 infusion and during their participation in 270-201 must have their antiretroviral medication regimen approved by the Investigator and Medical Monitor prior to starting antiretroviral treatment.	1

Section No./Title	Revision	Rationale
9.4.9/Treatment Compliance	Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on ~~the~~a dispensing log ~~provided for the study~~. Sites will be instructed to return or destroy all used and unused study drug containers.	14
9.5/IP Accountability	The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data, if allowed by local SOPs.	14
9.5.1/Return and Disposition of Clinical Supplies	Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials~~.~~ (or must be referenced in their institution SOPs).	14
9.6/Dietary or Other Protocol Restrictions	Subjects should be advised to abstain from any blood or sperm donation after BMN 270 infusion, until there is no further evidence of vector shedding from PCR analysis of samples.	10
9.7.2/Primary Efficacy Variables	Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52, as applicable and at the discretion of the Investigator.	3
9.7.3/Secondary Efficacy Variables	In subjects who experience recurrent bleeding episodes, the Investigator and Medical Monitor will discuss whether to resume prior FVIII prophylaxis.	5
Table 9.7.8.2.1	*Table 9.7.8.2.1 (and its footnotes) have been updated to reflect changes made elsewhere in the protocol.*	6
9.7.8.3/Liver and Hepatitis Testing	Evidence of ongoing hepatitis B or hepatitis C infection is exclusionary. Subjects ~~who have cleared~~with a history of hepatitis B ~~infection~~ or ~~are seronegative do not need to receive the~~ hepatitis ~~B vaccination.~~ C will be asked for information about the treatments received as part of their medical history assessment at Screening.	4
9.7.8.5/Vital Signs, Physical Examinations and Other Safety Observations	A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and ~~every 52 weeks thereafter; at other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.~~ at the End of	2, 8, 14

Section No./Title	Revision	Rationale
	Years visits thereafter. A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems. At other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, dermatologic, musculoskeletal, and gastrointestinal assessments. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses. Height will be recorded at Screening only. Weight will be recorded at Screening, at Weeks 26 and 52 during Year 1, and then at the second Q12W visit each year and at every ~~6 months thereafter~~End of Years visit during Years 2-5.	
9.7.8.6/Vector Shedding	Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor). Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule (refer to Section 12.5.3) but who have not cleared vector shedding from all fluids must still provide samples for assessment until vector shedding has cleared: every 4 weeks (during Year 2), or every 6 weeks (during Years 3-5). Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples~~.~~ (upon consultation between the Investigator and Medical Monitor).	3, 7, 14
10.1.1/Adverse Events	~~Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the subject is a haemophiliac) should not be recorded as AEs but will instead be captured in subject diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of major trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.~~	14

Section No./Title	Revision	Rationale
10.1.1.1/Bleeding and Suspected Bleeding Events	All bleeding events and suspected bleeding events, regardless of the need for exogenous FVIII therapy as treatment, should be captured in subject diaries and recorded on the designated bleeding eCRF. Bleeding events and suspected bleeding events should not be reported as adverse events, with the following exception: • All bleeding events and suspected bleeding events which meet one or more of the criteria for being serious (refer to Section 10.2) should be reported as serious adverse events (whether or not they are bleeding events that are normal sequelae of hemophilia, and whether or not they required exogenous FVIII as treatment).	14
10.2.1/Events of Special Interest	The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality: • Elevation of ~~liver enzymes (~~ALT~~)~~ ≥ 1.5x ULN, regardless of whether that triggers an initiation or modification of corticosteroid treatment • Thromboembolic event	14
10.4.1.4/Abnormal Laboratory Values	A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met: • ~~Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)~~	14
10.4.1.7/Hospitalization, Prolonged Hospitalization, or Surgery	There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to: • ~~Hospitalization solely for the purpose of insertion of~~Insert an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug ~~will not be considered an SAE~~or FVIII replacement therapy.	14
10.4.1.9/Pregnancy	Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up ~~Form in the study reference materials.~~eCRF.	14
10.9/BioMarin Contact Information	Contact information for the medical monitor is as follows:	13

Section No./Title	Revision	Rationale
	~~Name: Mitra Tavakkoli, MD, PharmD~~ ~~Address: 105 Digital Drive~~ ~~Novato, CA 94949 USA~~ ~~Phone: +1 (415) 257-5974~~ ~~E-mail: fatemeh.tavakkoli@bmrn.com~~ Name: Nina Mitchell, MA MB BChir MSc Address: Biomarin (UK) Ltd. 10 Bloomsbury Way London WC1A 2SL Phone: +44 (0) 207 420 3414 (office) +44 (0) 7761 659 535 (mobile) E-mail: nina.mitchell@bmrn.com	
12.2/Screening Visit	The following procedures will be performed during the Screening Period: • Full medical history, including haemophilia A history, hepatitis B, hepatitis C, and HIV. Subjects with a history of hepatitis B or hepatitis C will be asked for information about the treatments received. Any prior pharmacokinetics information obtained while the subject was receiving prophylactic or on-demand FVIII therapy prior to the study should also be collected. For subjects who have already enrolled in 270-201, this information should be collected at the next regularly scheduled study visit (or at least prior to the subject's completion of study participation).	4
12.8/Safety Follow-Up (Years 2-5)	Subjects who do not respond to BMN 270 treatment (ie, treatment failure, manifesting as either failure to achieve FVIII activity ≥ 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, follow an abbreviated visit schedule after Week 52 of the study by attending only the Q12W and End of Year visits during Years 2-5. Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until vector shedding has cleared (but do not have to perform other scheduled assessments at those study visits).	3, 7

Section No./Title	Revision	Rationale
12.8.1/Year 2 – Every 4 Weeks	During Year 2, every 4 weeks ~~(+~~(± 2 weeks), the following procedures will be performed: ~~• Physical examination~~ ~~○ Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.~~ ~~• Vital Signs~~ ~~• Exploratory biomarker assessments~~ • PCR of vector DNA in blood, saliva, urine, semen, and stools (if required) ○ Sample testing during Year 2 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks during Year 2 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor). ○ Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 4 weeks during Year 2 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).	2, 14
12.8.2/Years 3-5 – Every 6 Weeks	During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed: ~~• Physical examination~~ ~~○ Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.~~ ~~• Vital Signs~~ ~~• Exploratory biomarker assessments~~ • PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)	2, 7, 14

Section No./Title	Revision	Rationale
	○ Sample testing during Years 3-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52 and/or Year 2. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 6 weeks during Years 3-5 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor). ○ Subjects who meet the definition of treatment failure and wish to follow an abbreviated schedule but who have not cleared vector shedding from all fluids must still provide samples for assessment every 6 weeks during Years 3-5 until vector shedding has cleared (in such a case, these subjects do not need to perform other assessments except for PCR sample delivery scheduled at these timepoints).	
12.8.3/Years 2-5 – ~~Every 3 Months~~ Every 12 Weeks and End of Year Visits	~~During Years 2-5, every 3 months (± 2 weeks), the following procedures will be performed:~~ During Years 2-5, subjects will be asked to return to the study site for visits at the following study weeks (± 2 weeks): • Year 2 – Week 64, Week 76, Week 88, Week 104 • Year 3 – Week 116, Week 128, Week 140, Week 156 • Year 4 – Week 168, Week 180, Week 192, Week 208 • Year 5 – Week 220, Week 232, Week 244, Week 260 For each of these years, the last study visit listed (Week 104, Week 156, Week 208, and Week 260) will serve as an End of Year visit. At the every 12 week and End of Year visits, the following procedures will be performed: • Brief physical examination (at every 12 week visits) or complete physical examination (at End of Year visits) • Weight (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only) • Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) • Liver Tests (refer to Table 9.7.8.3.1) – local laboratory assessment ○ LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.	2, 3, 7, 11, 14

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	• FVIII activity level – local laboratory assessment
	  ○ FVIII activity level (one-stage clotting assay)
	  ○ FVIII activity level (chromogenic substrate assay)
	• Liver Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
	  ○ LTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
	• FVIII Assays – central laboratory assessment
	  ○ FVIII activity level (one-stage clotting assay)
	  ○ FVIII activity level (chromogenic substrate assay)
	  ○ FVIII coagulation activity exploratory assay
	  ○ Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
	  ○ FVIII antigen (ELISA)
	Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
	• Vital Signs
	• Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
	  ○ ABO blood typing assessment should be performed at the next regularly scheduled study visit (or at least prior to the end of the subject's participation in the study)
	• Exploratory biomarker assessments
	• Haemo-QoL-A QOL assessment (at Week 76, Week 128, Week 180, Week 232, and End of Year visits only)
	• PCR of vector DNA in blood, saliva, urine, semen, and stools (if required)	

Section No./Title	Revision	Rationale
	○ Sample testing during Years 2-5 is not required if at least 3 consecutive samples are negative during the Post-Infusion Follow-Up period in Weeks 1-52. Subjects who have not had 3 consecutive negative semen samples by Week 52 should continue to have PCR testing of semen every 4 weeks (during Year 2) or every 6 weeks (during Years 3-5) until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).	
~~12.8.4/Years 2-5 – Every 6 Months~~	~~Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedures will be performed:~~ • ~~Haemo-QoL-A QOL assessment~~ • ~~Weight~~	2
~~12.8.5/Years 2-5 – Every 4 Weeks~~	~~Every four weeks during Years 2-5, the following procedure may be performed:~~ • ~~PCR of vector DNA in blood, saliva, urine, semen, and stools~~ ○ ~~Sample testing during Years 2-5 is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks during Years 2-5 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).~~	2

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI , MD, PharmD PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Subject Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015
Date of Amendment 4:	02 September 2016
Date of Amendment 5:	14 February 2017
Date of Amendment 6:	21 December 2017

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 6

Date: 21 December 2017

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 6 to the BMN 270-201 protocol is provided below.

1. The frequency of liver function and FVIII testing has been increased during the Years 2-5 follow-up period. Testing will be performed every 4 weeks (+ 2 weeks) during year 2, and every 6 weeks (± 2 weeks) during years 3-5.

Rationale: To maintain adequate surveillance of potential hepatocyte injury and trending of FVIII activity beyond Week 52 post-BMN 270 infusion, the frequency of liver function and FVIII testing has been increased during the Years 2-5 follow-up period from every 3 months beyond Week 52. A brief physical examination, assessment of vital signs, and review of adverse events, concomitant medications, and diaries of FVII usage and bleeding events will also occur at these visits.

2. Exploratory biomarker sampling has been extended beyond Week 24. Samples will now be collected every 4 weeks through the end of Year 2, and then every 6 weeks during Years 3-5.

Rationale: Additional biomarker sampling will permit further analysis of exploratory endpoints during the study.

3. Clarified that the 72-hour washout period prior to assessment of FVIII levels is necessary only for subjects who have achieved FVIII levels ≥ 5 IU/dL by Week 16 after BMN 270 infusion and have not resumed standard of care FVIII replacement therapy.

Rationale: The current language in the protocol states: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved." The proposed language is to make it more clear that the 72 hour wash-out period prior to FVIII assessment is not intended for the subjects who have resumed standard of care FVIII replacement therapy.

4. Clarified that testing for reactivation of hepatitis B and hepatitis C at Week 16 is necessary only in subjects who have a past medical history of hepatitis B or hepatitis C prior to study entry.
5. Minor administrative changes have been made for consistency and clarity.

6. The identity of the medical monitor has been updated.

Specific changes included in this amendment, including the Synopsis, since Amendment 5 (approved 14 February 2017) are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced

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dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 1).

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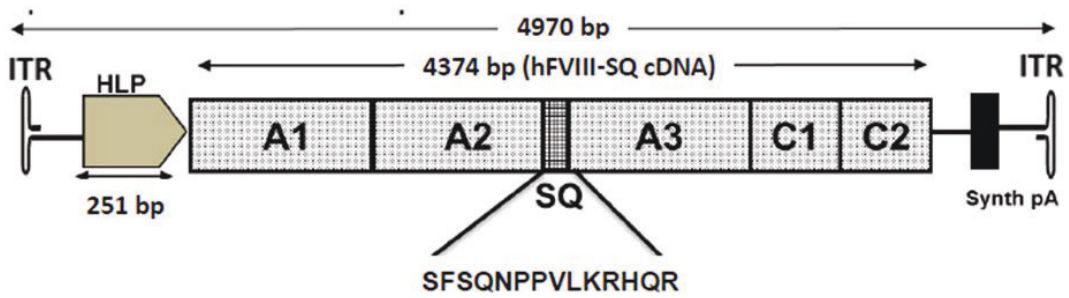
Figure 1. hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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

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STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)

Cohort 2: 2E13 vg per kilogram, iv

Cohort 3: 6E13 vg per kilogram, iv

Cohort 4: 4E13 vg per kilogram, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in non-human primates.

Cohorts 1-3

The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the one-stage aPTT and chromogenic assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

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If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

NUMBER OF SUBJECTS PLANNED:

Up to 15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

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1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A

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7. Platelet count of < 100 x 10^9/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous infusion.

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CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver function tests (LFTs, including ALT, AST, GGT, LDH, bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LFTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52) during Year 1. LFTs will be monitored every 4 weeks during Year 2, and then every 6 weeks for Years 3-5 post-dose in the safety extension; the frequency and duration of LFT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

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If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay at 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as vector shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
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
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
HA	Haemophilia A
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee
IND	Investigational New Drug (application)

INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LFT	liver function test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver function tests (LFTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LFTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman, 2013, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay, 2012, Blood).

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2013, Haemophilia.); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This

nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in

the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani, 2006, Blood). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Engl.J.Med.), (Nathwani, 2014, N.Engl.J.Med.). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola, 2014, J.Hepatol.). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Mannucci, 2001, N.Engl.J.Med.). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

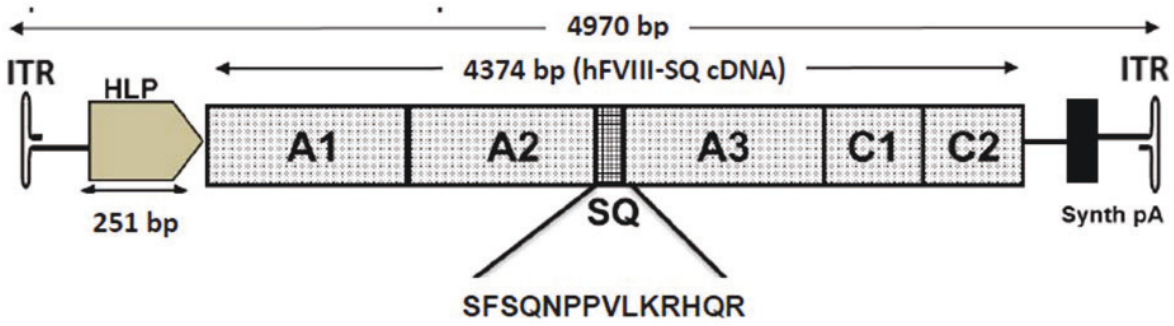
Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011, Haemophilia.). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia.), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci, 2001, N.Engl.J.Med.). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol.Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, 2014, N.Engl.J.Med.). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.), (Bainbridge, 2008, N.Engl.J.Med.), (Maguire, 2009, Lancet); (Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan (Nathwani, 2011, Mol.Ther.). Each subject will have a comprehensive surveillance plan that monitors LFTs during the study.

For additional information on safety findings in 270-201, refer to current version of the Investigator's Brochure.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related

changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

- Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
- Cohort 2: 2E13 vg per kilogram, iv
- Cohort 3: 6E13 vg per kilogram, iv
- Cohort 4: 4E13 vg per kilogram, iv

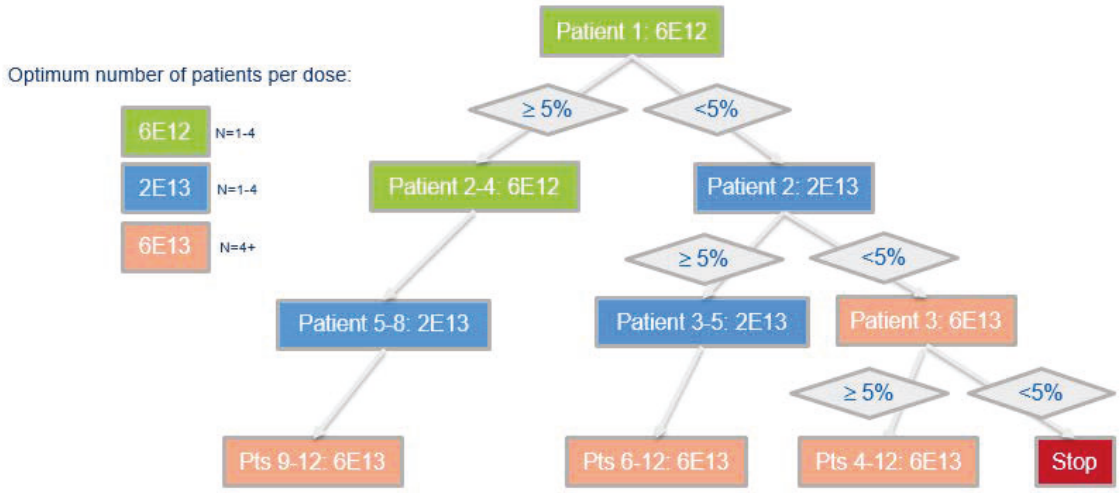
The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a 10-fold safety margin from no observed adverse effect level (NOAEL) in mice.

Cohorts 1-3

The first three cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches ≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme for Cohorts 1 to 3



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the Data Review Board (DRB). The adaptive nature of this trial allows the DRB

to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected PBMC. Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the DRB and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 and Table 9.1.4 for Safety Follow-up.

Table 9.1.5, dealing with therapeutic corticosteroid use in the event of elevated LFTs, is discussed in Section 9.4.8.2.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	Smart Rescreening[i] (Day -28 to Day -1)	Baseline (Day -7 to Day -1)[h]	
Informed consent	X			
Medical History	X			
Physical Examination[a]	X		X	X
Height and Weight[a]	X			
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, coagulation screen, and CRP[e]	X	X	X	
Urine Tests[e]	X	X	X	
Liver Function Tests[e]	X	X	X	
PBMC collection for CTL baseline			X	
Von Willebrand Factor Antigen (VWF:Ag)	X			
Direct Thrombin Test			X	
PCR of vector DNA in blood, saliva, urine, semen, and stools			X	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

[b] Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). Sample collection on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples will be performed only as deemed necessary by the Sponsor.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, LFTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion must be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up (Week 1-16)

Assessment	Follow-Up After BMN 270 Administration – Weeks*																
	Week 1																
	D2	D4	D8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical examination[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X		X				X
Urine Tests[b]						X				X				X				X
Liver Function Tests (local)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Function Tests (central)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer						X				X				X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X	X	X			X		X		X		X		X		X		X
Exploratory biomarker assessments[f]						X				X				X				X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C																		X[g]
PBMC collection			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Von Willebrand Factor Antigen (VWF:Ag)						X				X				X				X
Direct Thrombin test			X												X			

* Visit windows are ± 48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then until at least 3 consecutive negative results are obtained. Collection and testing of semen samples must continue through Week 12, even if 3 consecutive negative samples in that compartment have already been recorded.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples will be performed only as deemed necessary by the Sponsor.

[g] Testing for reactivation of hepatitis B and hepatitis C at Week 16, for subjects with a past medical history of Hepatitis B or Hepatitis C prior to study entry, should be performed only in subjects who have not received therapeutic corticosteroids prior to Week 16; subjects who have received therapeutic corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.5.

Table 9.1.3: Schedule of Events – Safety Follow-Up (Week 17-32)

Assessment	Follow-Up After BMN 270 Administration – Weeks*															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight										X						
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X				X				X				X
Urine Tests[b]				X				X				X				X
Liver Function Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Function Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer				X								X				
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X				X				X				X
Exploratory biomarker assessments[f]				X				X				X				X
Haemo-QoL-A QoL assessment												X				
AAV5 antibody titer				X				X				X				X
PBMC collection	X	X	X	X		X		X		X		X		X		X
Von Willebrand Factor Antigen (VWF:Ag)				X				X				X				X
Direct Thrombin test										X						

* Visit windows are ± 48 hours

[a] Brief physical examination should be done at all weekly visits. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples will be performed only as deemed necessary by the Sponsor.

Table 9.1.4: Schedule of Events – Safety Follow-Up

	Year 1 – Weeks*												Years 2-5*	Year 2*	Years 3-5*	ETV
Assessment	**33**	**34**	**35**	**36**	**38**	**40**	**42**	**44**	**46**	**48**	**50**	**52**	**Q3M**	**Q4W**	**Q6W**	
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Weight[a]												X	X[a]			
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X	X			X
Urine Tests[b]				X		X		X		X		X	X			X
Liver Function Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Liver Function Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
AAV5 antibody titer				X		X		X		X		X	X			X
FVIII antibody titer				X				X				X	X			X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)		X		X				X				X	X			X
Von Willebrand Factor Antigen (VWF:Ag)				X		X		X		X		X	X			X
Direct Thrombin Test					X							X	X			X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X		X		X		X		X	X[e]			X
Exploratory biomarker assessments[g]				X		X		X		X		X		X	X	X
Haemo-QoL-A QoL assessment												X	X[f]			X

[*] Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52 and ± 2 weeks for visits in Years 2-5. For LFT, FVIII, and exploratory biomarker testing during Years 2-5, the visit windows are every 4 weeks (+ 2 weeks) during Year 2, and every 6 weeks (±2 weeks) during Years 3-5.

[a] Complete physical examination should be performed at Week 52 and every 52 weeks thereafter; brief physical examination may be performed at other study visits. Refer to Section 9.7.8.5. During Years 2-5, weight should be performed every 6 months.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) in years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

[f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).

[g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples will be performed only as deemed necessary by the Sponsor.

Table 9.1.5: Schedule of Events – Therapeutic Corticosteroids

	Steroid Treatment Period[d]											Post-Steroid Period[c]				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11[b]**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 13**
Therapeutic corticosteroids (dose in mg/day) [a]	60 mg	60 mg	40 mg	40 mg	30 mg	30 mg	20 mg	20 mg	15 mg	10 mg	5 mg					
FVIII activity testing												X	X	X	X	
Liver function testing												X	X	X	X	
Hepatitis B testing[e]						X						X				X
HCV Viral Load[e]						X						X				X

[a] Therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor.

[b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

[c] After discontinuation of corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. If these assessments are already being done as part of normal study follow-up, they do not need to be duplicated.

[d] Regardless of the assessments listed in the Schedule of Assessments (Table 9.1.2, Table 9.1.3, or Table 9.1.4), subjects initiated on corticosteroids will only be required to have laboratory evaluations on a weekly basis.

[e] Should only be performed in subjects with a history of hepatitis B or hepatitis C prior to study entry.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4.
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- Any ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270, in the absence of a definitive alternate etiology for the increase
- The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion
- The detection of AAV vector DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than 52 weeks after BMN 270 infusion, as discussed in Section 9.7.8.6
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt:

- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days

9.3.4 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.5 Duration of Subject Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical examination performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (e.g. the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be

discharged from the clinic unless toxicity has been observed in which case the stay in the clinic may be extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

For Cohorts 1 to 3, approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1)

at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in that cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design for Cohorts 1-3

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents

Medications which are predominately metabolized by the liver (e.g., acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LFTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52). LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

9.4.8.2.1 Therapeutic Corticosteroids

In general, therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator.

Reports of raised LFTs (defined as ALT ≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

Following initiation or completion of therapeutic corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

Treatment with prednisolone will be initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.5).

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

Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. For the subjects with a past medical history of hepatitis B or hepatitis C prior to study entry, Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin

and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a study drug return form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1 through Table 9.1.4) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to expression of FVIII at 5 IU/dL or above, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes (Den Ujil, 2011, Haemophilia).

The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic FXa assay)
- FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time)

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII

≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

The FVIII activity level in both assays and the number of subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.

FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4, but will be used in conjunction with local lab LFT assessments to monitor subject safety and need for initiation of therapeutic corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage APTT and/or chromogenic FXa assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.6 Exploratory Assessments

Blood samples will be collected at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed.

On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with samples collected specifically for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	Haematology	Urine Tests	Other
Albumin	Haemoglobin	Appearance	CRP
BUN	Haematocrit	Color	
Calcium	WBC count	pH	**Coagulation Screen including:**
Chloride	RBC count	Specific gravity	APTT
Total cholesterol	Platelet count	Ketones	PT/INR
CO_2	Differential cell count	Protein	TT
CPK		Glucose	
Creatinine		Bilirubin	
Glucose		Nitrite	
Phosphorus		Urobilinogen	
Potassium		Haemoglobin	
Total protein			
Sodium			
Uric Acid			

BUN, blood urea nitrogen; CO_2, carbon dioxide; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

9.7.8.3 Liver Function and Hepatitis Testing

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active hepatitis B or hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing hepatitis B or hepatitis C infection is exclusionary. Subjects who have cleared a hepatitis B infection or are seronegative do not need to receive the hepatitis B vaccination.

Subjects with a history of hepatitis B or hepatitis C infection prior to study entry will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.

A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopoenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

Liver function tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4. At each time point, the following LFTs should be assessed:

Table 9.7.8.3.1: Liver Function Tests

Liver Function Tests			
Alkaline Phosphatase	AST (SGOT)	Total Bilirubin	LDH
ALT (SGPT)	Direct Bilirubin	GGT	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase

Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

9.7.8.4 HIV Testing

HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.

9.7.8.5 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every

15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and every 52 weeks thereafter; at other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems.

A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, and gastrointestinal assessments.

Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

9.7.8.6 Vector Shedding

Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid but not in motile sperm (Manno, 2006, Nat.Med.). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016, Haemophilia).

During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of vector shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the subject is a haemophiliac) should not be recorded as AEs but will instead be captured in subject diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of major trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening

  Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (e.g. anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of liver enzymes (ALT) that triggers an initiation or modification of corticosteroid treatment

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.2. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE (or SAE, as appropriate) eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. Events that change in severity necessitate the recording of an additional AE. AEs that do not have a change in severity should be recorded only once on the eCRF.

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE or EOSI should be reported as such, and recorded in the AE eCRF. Any laboratory result abnormality of CTCAE Grade 4 or 5 should be recorded as an SAE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death

is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site

becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the

investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax:
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI, MD, PharmD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
E-mail:

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic FXa assay and the one-stage APTT assay are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LFTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, hepatitis B, hepatitis C, and HIV.
- Complete Physical Examination
- Height and weight
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic FXa (plasma)
    - Baseline hFVIII activity level – one-stage APTT assay
    - hFVIII coagulation activity exploratory assay
    - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
    - hFVIII total antibody assay
    - hFVIII antigen (ELISA)

- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Screen for hepatitis B, hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)
- Von Willebrand Factor Antigen (VWF:Ag)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)

- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)
- PBMC collection for CTL baseline
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- BMN 270 Infusion
- Vital Signs

- Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Once per week (Weeks 1 through 16)

The following procedures will be performed at one visit per week from Weeks 1 through 16:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- Samples for FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
    - Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16

- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- PBMC collection

12.5.2 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only) – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – central laboratory assessment
- Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – local laboratory assessment
- FVIII activity level (Day 4 only) – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)

12.5.3 Week 1 – Day 8

On Day 8, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools

- Direct Thrombin test
- Haemo-QoL-A QoL assessment

12.5.4 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) (not assessed at Week 14)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2)
    - Collection to occur until at least 3 consecutive negative results are obtained. Semen samples should continue to be collected and tested through Week 12, even if 3 consecutive negative results in that compartment have been recorded prior to that timepoint.

12.5.5 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedures will be performed:

- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- FVIII antibody titer
- Exploratory biomarker assessments

12.5.6 Week 16

At Week 16, the following procedures will be performed:

- Test for hepatitis B and hepatitis C reactivation (in subjects with a history of hepatitis B or hepatitis C infection prior to study entry)
    - Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.

12.5.7 Weeks 8 and 16

At Weeks 8 and 16, the following procedures will be performed:

- AAV5 antibody titer

12.5.8 Weeks 2, 3, 4, and 16

At Weeks 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.5.9 Week 13

At Week 13, the following procedure will be performed:

- Direct Thrombin test

12.6 Safety Follow-Up – Weeks 17-36

After the Post-Infusion Follow-Up visits are complete, subjects will return to the study site for Safety Follow-Up visits from Weeks 17 through Week 36 (± 48 hours), when the following procedures will be completed:

12.6.1 Once per week (Weeks 17 through 36)

Once per week from Week 17 through Week 36, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment

- LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII activity level – local laboratory assessment
  - FVIII activity level (one-stage APTT)
  - FVIII activity level (chromogenic FXa assay)

12.6.2 Once per week (Weeks 17 through 20)

Once per week from Week 17 through Week 20, the following procedures will be performed:

- PBMC collection

12.6.3 Every 2 weeks (Weeks 21 through 36)

Every 2 weeks (Weeks 22, 24, 26, 28, 30, 32, 34, and 36), the following procedures will be performed:

- PBMC collection

12.6.4 Every 4 Weeks

Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- AAV5 antibody titer
- PCR of vector DNA in blood, saliva, urine, semen, and stools
  - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.5 Every 8 Weeks

Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed:

- FVIII antibody titer

12.6.6 Week 26

At Week 26, the following procedure will be performed:

- Direct Thrombin test

- Weight

12.6.7 Week 28

At Week 28, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Weeks 37-52

Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:

12.7.1 Once per visit

At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

12.7.2 Every 4 Weeks

Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed:

- Exploratory biomarker assessments
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.7.3 Every 8 Weeks

Every 8 weeks (Weeks 44 and 52), the following procedure will be performed:

- PBMC collection
- FVIII antibody titer

12.7.4 Week 38 and 52

At Week 38 and Week 52, the following procedure will be performed:

- Direct Thrombin test

12.7.5 Week 52

At Week 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment
- Weight

12.8 Safety Follow-Up – Years 2-5

During Years 2-5 of Safety Follow-up, the following procedures will be completed:

12.8.1 Year 2 – Every 4 Weeks

During Year 2, every 4 weeks (+ 2 weeks), the following procedures will be performed:

- Physical examination
    - Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (FXa chromogenic assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.

- Exploratory biomarker assessments

12.8.2 Years 3-5 – Every 6 Weeks

During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed:

- Physical examination
    - Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (FXa chromogenic assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- Exploratory biomarker assessments

12.8.3 Years 2-5 – Every 3 Months

During Years 2-5, every 3 months (± 2 weeks), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test

12.8.4 Years 2-5 – Every 6 Months

Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedures will be performed:

- Haemo-QoL-A QOL assessment
- Weight

12.8.5 Years 2-5 – Every 4 Weeks

Every four weeks during Years 2-5, the following procedure may be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Years 2-5 is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks during Years 2-5 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.9 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 260 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical examination

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Exploratory biomarker assessment
- Haemo-QoL-A QOL assessment

12.10 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 260). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

14.2 Missing data will not be imputed.

14.3 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay as measured by the central laboratory at 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.4 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.5 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma

profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.6 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.7 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.8 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.9 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon

completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572 and/or principles of ICH E6 R2 GCP, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and/or ICH E6 R2 Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/ IEC review and approval in 21 CFR Part 56 and/or ICH E6 R2 Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH E6 R2 Section 4.11.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH E6 R2 Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH E6 R2 Section 4.9.7.
- The IRB/IEC/REB complies with the requirements of 21 CFR Part 56, ICH E6 R2 Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201, Amendment 6

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including ICH E6 R2, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

DocuSigned by:
PI

Medical Monitor Signature Date

Printed name: PI , MD, PharmD, PI _____

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See pages 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Text Revisions	Rationale
Synopsis/Study Design and Plan	The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a ~~significant~~10-fold safety margin ~~(10-fold)~~ from no observed adverse effect level (NOAEL) in non-human primates.	5
Synopsis/Criteria for Evaluation	LFTs will be monitored every ~~three months~~4 weeks during Year 2, and then every 6 weeks for ~~up to~~ Years 3-5 ~~years~~ post-dose in the safety extension; the frequency and duration of LFT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.	1
9.1/Overall Study Design and Plan	The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a ~~significant~~10-fold safety margin ~~(10-fold)~~ from no observed adverse effect level (NOAEL) in non-human primates.	5
Table 9.1.1 (Footnotes)	[g] Blood samples will be collected ~~from subjects~~ to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to ~~Haemophilia~~ hemophilia A ~~disease.~~, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing ~~on~~ of these samples ~~is optional~~ will be performed only as deemed necessary by the Sponsor.	5
Table 9.1.2 (Footnotes)	[c] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. [d] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who	3, 4, 5

Section No./Title	Text Revisions	Rationale
	have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. [f] Blood samples will be collected ~~from subjects~~ to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to ~~Haemophilia~~ hemophilia A ~~disease.~~ , coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing ~~on~~ of these samples ~~is optional~~ will be performed only as deemed necessary by the Sponsor. [g] Testing for reactivation of hepatitis B and hepatitis C at Week 16, for subjects with a past medical history of Hepatitis B or Hepatitis C prior to study entry, should be performed only in subjects who have not received therapeutic corticosteroids prior to Week 16; subjects who have received therapeutic corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.5.	
Table 9.1.3	Table 9.1.3 has been updated to be consistent with changes made elsewhere in the protocol.	2
Table 9.1.3 (Footnotes)	[c] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. [d] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. [f] Blood samples will be collected ~~from subjects~~ to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to ~~Haemophilia~~ hemophilia A ~~disease.~~ , coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be	2, 3, 5

Section No./Title	Text Revisions	Rationale
	associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing ~~on~~ of these samples ~~is optional~~ will be performed only as deemed necessary by the Sponsor.	
Table 9.1.4	Table 9.1.4 has been updated to be consistent with changes made elsewhere in the protocol.	1, 2, 3
Table 9.1.4 (Footnotes)	* Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52 and ± 2 weeks for visits in Years 2-5. For LFT, FVIII, and exploratory biomarker testing during Years 2-5, the visit windows are every 4 weeks (+ 2 weeks) during Year 2, and every 6 weeks (±2 weeks) during Years 3-5. [c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. [d] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved~~.~~; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism. If a subject tests positive in the Bethesda assay (with Nijmegen modification) in years 3-5, a fresh sample should be collected within 4 weeks of the initial visit that had a positive result. [g] Blood samples will be collected to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A. While exploratory samples will be collected at the time points indicated above, testing of these samples will be performed only as deemed necessary by the Sponsor.	1, 2, 3, 5
Table 9.1.5 (Footnotes)	[e] Should only be performed in subjects with a history of hepatitis B or hepatitis C prior to study entry.	4

Section No./Title	Text Revisions	Rationale
9.4.8.2.1/Therapeutic Corticosteroids	For subjects with a past medical history of hepatitis B or hepatitis C prior to study entry, Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment.	4
9.7.2/Primary Efficacy Variable	If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.	3
9.7.6/Exploratory Assessments	Blood samples will be collected ~~from subjects~~ at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to ~~Haemophilia A disease. The exploratory genetic/genomic testing is optional.~~ hemophilia A, coagulation, and/or AAV gene transfer, and to develop assays used for these evaluations. Subjects may choose to opt out of the exploratory genetic/genomic research being done to study or try to discover genes that are not yet known to be associated with hemophilia A.	5
9.7.8.3/Liver Function and Hepatitis Testing	Subjects with a history of hepatitis B or hepatitis C infection prior to study entry will be tested for hepatitis B and hepatitis C reactivation at Week 16.	4
10.4.1.4/Abnormal Laboratory Values	Any laboratory result abnormality fulfilling the criteria for a SAE or EOSI should be reported as such, and recorded in ~~addition to being~~ the AE eCRF. Any laboratory result abnormality of CTCAE Grade 4 or 5 should be recorded as an ~~AE~~SAE in the AE eCRF.	5
10.9/BioMarin Contact Information	Contact information for the medical monitor is as follows: Name: ~~Liron Walsh~~Mitra Tavakkoli, MD, PharmD Address: 105 Digital Drive Novato, CA 94949 USA Phone: +1 (415) ~~455-7870~~257-5974 E-mail: ~~liron.walsh~~fatemeh.tavakkoli@bmrn.com	5
12.5.1/Once per Week (Weeks 1-16)	The following procedures will be performed at one visit per week from Weeks 1 through 16: • Samples for FVIII Assays – central laboratory assessment	6

Section No./Title	Text Revisions	Rationale
	Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.	
12.5.1/Week 1 – Day 2 and Day 4	On Day 2 and Day 4 of Week 1, the following procedures will be performed: • Samples for FVIII Assays (Day 4 only) – central laboratory assessment Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.	3
12.5.6/Week 16	At Week 16, the following procedures will be performed: • Test for hepatitis B and hepatitis C reactivation (in subjects with a history of hepatitis B or hepatitis C infection prior to study entry) o ~~Subjects will be tested for hepatitis B and hepatitis C reactivation at Week 16.~~ Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.	4, 5
12.6.1/Once per Week (Weeks 17 through 36)	Once per week from Week 17 through Week 36, the following procedures will be performed: • FVIII Assays – central laboratory assessment Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.	3
12.6.4/Every 4 Weeks	Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed: • Exploratory biomarker assessments ~~(Weeks 20 and 24 only)~~	2

Section No./Title	Text Revisions	Rationale
12.7.1/Once per visit	At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed: • FVIII Assays – central laboratory assessment Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.	3
12.7.2/Every 4 Weeks	Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed: • Exploratory biomarker assessments	2
12.8/Safety Follow-Up	During Years 2-5 of Safety Follow-up, ~~subjects will be assessed every 3 months (± 2 weeks). At these times,~~ the following procedures will be completed:	5

12.8.1/Year 2 – Every 4 Weeks	During Year 2, every 4 weeks (+ 2 weeks), the following procedures will be performed:
	• Physical examination
	  ○ Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.
	• Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
	• Vital Signs
	• Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
	  ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
	• FVIII activity level – local laboratory assessment
	  ○ FVIII activity level (one-stage APTT)
	  ○ FVIII activity level (chromogenic FXa assay)
		1, 2, 3, 5

Section No./Title	Text Revisions	Rationale

	• Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
	  ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
	• FVIII Assays – central laboratory assessment
	  ○ FVIII activity level (one-stage APTT)
	  ○ FVIII activity level (FXa chromogenic assay)
	  ○ FVIII coagulation activity exploratory assay
	  ○ Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
	  ○ FVIII antigen (ELISA)
	Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy.
	• Exploratory biomarker assessments	

12.8.2/Years 3-5 – Every 6 Weeks	During Years 3-5, every 6 weeks (± 2 weeks), the following procedures will be performed:
	• Physical examination
	  ○ Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.
	• Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
	• Vital Signs
	• Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
	  ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
	• FVIII activity level – local laboratory assessment
		1, 2, 3, 5

Section No./Title	Text Revisions	Rationale
	○ FVIII activity level (one-stage APTT) ○ FVIII activity level (chromogenic FXa assay) • Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII Assays – central laboratory assessment ○ FVIII activity level (one-stage APTT) ○ FVIII activity level (FXa chromogenic assay) ○ FVIII coagulation activity exploratory assay ○ Bethesda assay (with Nijmegen modification) for FVIII inhibitor level ○ FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. • Exploratory biomarker assessments	
12.8.3/~~Every Visit~~ Years 2-5 – Every 3 Months	~~Every~~ During Years 2-5, every 3 months (± 2 weeks), the following procedures will be performed: • ~~Physical examination~~ ○ ~~Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.~~ • ~~Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)~~ • ~~Vital Signs~~ • ~~Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment~~	1, 3, 5

Section No./Title	Text Revisions	Rationale
	~~○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.~~ ~~• FVIII activity level – local laboratory assessment~~ ~~○ FVIII activity level (one-stage APTT)~~ ~~○ FVIII activity level (chromogenic FXa assay)~~ ~~• Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment~~ ~~○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.~~ ~~• FVIII Assays – central laboratory assessment~~ ~~○ FVIII activity level (one-stage APTT)~~ ~~○ FVIII activity level (FXa chromogenic assay)~~ ~~○ FVIII coagulation activity exploratory assay~~ ~~○ Bethesda assay (with Nijmegen modification) for FVIII inhibitor level~~ ~~○ FVIII antigen (ELISA)~~ ~~Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.~~	
12.8.4/~~Every Other Visit~~ Years 2-5 – Every 6 Months	Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following ~~procedure~~ procedures will be performed:	5
12.8.5/Years 2-5 – Every 4 Weeks	Every four weeks during Years 2-5, the following procedure may be performed: • PCR of vector DNA in blood, saliva, urine, semen, and stools ○ Sample testing during Years 2-5 is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks during Years 2-5 until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).	5

Section No./Title	Text Revisions	Rationale
12.9/Early Termination Visit	At the Early Termination visit, as many of the following assessments as possible should be done: • FVIII Assays – central laboratory assessment Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved; the 72-hour wash-out period is only intended for subjects who have achieved FVIII ≥ 5 IU/dL at 16 weeks after BMN 270 infusion and who have not resumed standard of care FVIII replacement therapy. • Exploratory biomarker assessment	2, 3
22.1/Conduct of Study and Protection of Human Patients	In accordance with FDA Form 1572 and/or principles of ICH E6 R2 GCP, the Investigator will ensure that: • He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and/or ICH E6 R2 Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/ IEC review and approval in 21 CFR Part 56 and/or ICH E6 R2 Section 2.6 are met. • He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH E6 R2 Section 4.11. • ~~He or she will ensure that~~ Adequate and accurate records in accordance with 21 CFR 312.62 and ~~to make~~/or ICH E6 R2 Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68~~.~~ • ~~He~~ and/or ~~she will ensure that~~ ICH E6 R2 Section 4.9.7. • The IRB/IEC/REB complies with the requirements of 21 CFR Part 56, ICH E6 R2 Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients. • He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.	5

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI , MD PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Subject Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015
Date of Amendment 4:	02 September 2016
Date of Amendment 5:	14 February 2017

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 5

Date: 14 February 2017

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 5 to the BMN 270-201 protocol is provided below.

1. Twice weekly testing (at the local laboratories) of FVIII and ALT levels during Weeks 1-20 has been reduced to once weekly testing.

Rationale: Current data from subjects enrolled in the study support a reduced frequency of local ALT and FVIII assessments in order to maintain adequate surveillance of hepatocyte injury and FVIII activity. The accumulated data suggest that week-to-week increases in ALT (if observed) are mild, and as such timely administration of corticosteroids will not be impacted by a reduction to once weekly testing. As already stipulated in the protocol, an observed increase in ALT, or decline in FVIII activity, may require an adjustment to the monitoring schedule as needed based on individual subject data (ie, increased monitoring in case of increases in ALT) and upon discussion between the Investigator and the Medical Monitor.

2. The frequency of PBMC collection has been increased to weekly through Week 20, then every other week through Week 36.

Rationale: Previous FIX gene-therapy clinical studies have demonstrated an association between increases in ALT and declines in FIX expression, which are thought to be due to a cell-mediated immune response to transduced hepatocytes (Manno, 2006, Nat.Med.) (Mingozzi, 2013, Blood); (Nathwani, 2014, N.Engl.J.Med.); (Nathwani, 2011, Mol.Ther.). An analysis of this immune response requires the collection of peripheral blood mononuclear cells (PBMC), which is part of the current schedule of assessments. The transient nature of the reported data indicates we may need to sample more frequently to identify a potential cell-mediated immune response to transduced hepatocytes. The frequency of PBMC collection has been increased from every 4 weeks to weekly through Week 20, then to every 2 weeks through Week 36.

3. Subjects will be required to continue to provide semen samples for PCR analysis through Week 12, even if they have already had 3 consecutive negative results in semen prior to that timepoint.

Rationale: A monitoring period of at least 12 weeks is required for semen to conform with regulatory guidances for shedding analysis, which recommend monitoring for at least one complete cycle of spermatogenesis (mean: 74 days, 95% CI interval: 70-78 days) (Heller and Clermont, 1964; Heller, 1969). Collection and testing of semen for vector DNA will therefore be continued through Week 12, regardless of whether the semen fluid compartment is considered to have cleared (i.e., the subject has had 3 consecutive negative sample tests). This new minimal monitoring period should account for the potentially delayed appearance of vector DNA in semen if a sperm progenitor cell had been transduced.

4. Language has been added to allow exploratory fractionation of collected samples (such as plasma, PBMCs, and red blood cells).

Rationale: On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with a portion of samples designated for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.

5. Minor administrative changes have been made for consistency and clarity.

Specific changes included in this amendment, including the Synopsis, since Amendment 4 (approved 2 September 2016) are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced

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dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 1).

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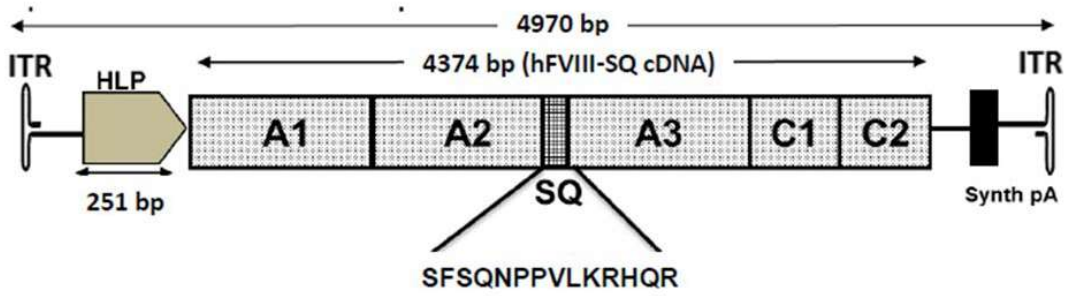
Figure 1. hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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

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- To assess the impact of BMN 270 on the number of bleeding episodes requiring treatment during the study

STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)

Cohort 2: 2E13 vg per kilogram, iv

Cohort 3: 6E13 vg per kilogram, iv

Cohort 4: 4E13 vg per kilogram, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Cohorts 1-3

The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the one-stage aPTT and chromogenic assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

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Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

NUMBER OF SUBJECTS PLANNED:

Up to 15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

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DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4

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5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous infusion.

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CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver function tests (LFTs, including ALT, AST, GGT, LDH, bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LFTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52) during Year 1. LFTs will be monitored every three months for up to 5 years post-dose in the safety extension; the frequency and duration of LFT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

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If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay at 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as vector shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
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
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
HA	Haemophilia A
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee
IND	Investigational New Drug (application)

INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LFT	liver function test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver function tests (LFTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LFTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman, 2013, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay, 2012, Blood).

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2013, Haemophilia.); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This

nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in

the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani, 2006, Blood). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Engl.J.Med.), (Nathwani, 2014, N.Engl.J.Med.). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola, 2014, J.Hepatol.). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Mannucci, 2001, N.Engl.J.Med.). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. . The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

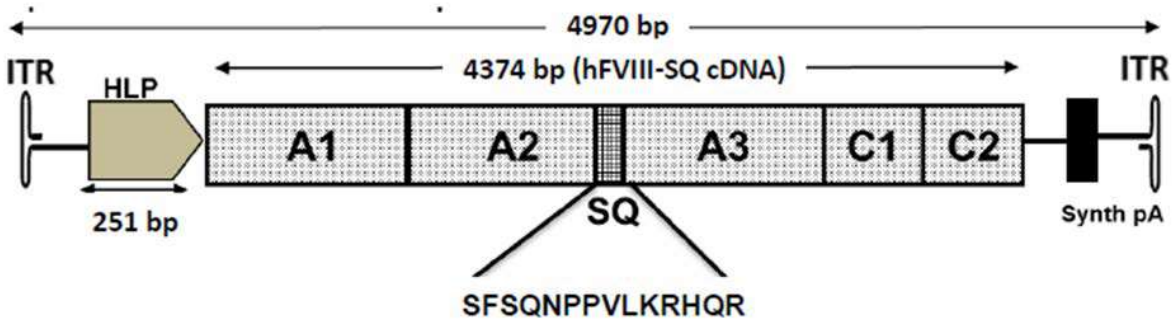
Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011, Haemophilia.). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia.), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci, 2001, N.Engl.J.Med.). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol.Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, 2014, N.Engl.J.Med.). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.), (Bainbridge, 2008, N.Engl.J.Med.), (Maguire, 2009, Lancet); (Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan (Nathwani, 2011, Mol.Ther.). Each subject will have a comprehensive surveillance plan that monitors LFTs during the study.

For additional information on safety findings in 270-201, refer to current version of the Investigator's Brochure.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related

changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

- Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
- Cohort 2: 2E13 vg per kilogram, iv
- Cohort 3: 6E13 vg per kilogram, iv
- Cohort 4: 4E13 vg per kilogram, iv

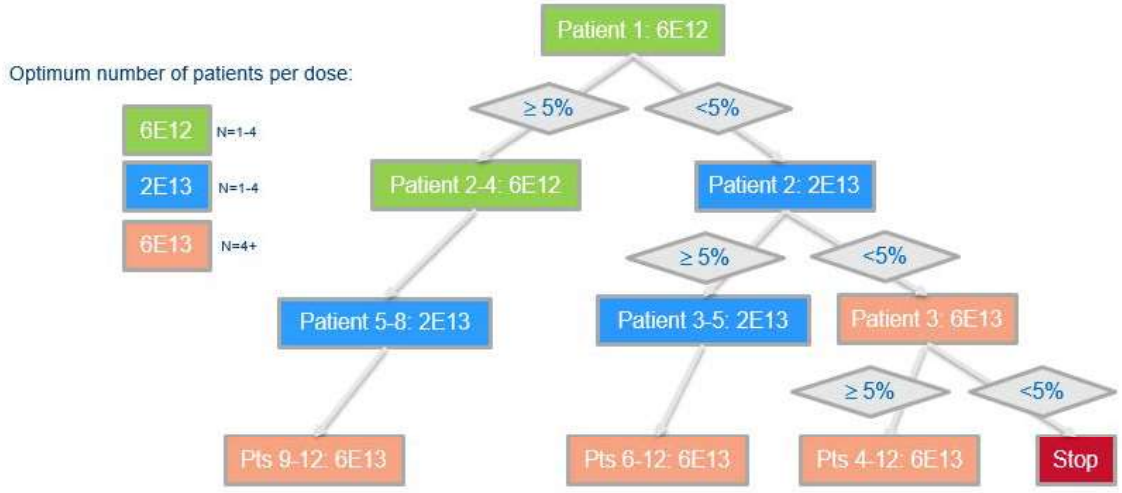
The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in mice.

Cohorts 1-3

The first three cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays.
Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches ≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme for Cohorts 1 to 3



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the Data Review Board (DRB). The adaptive nature of this trial allows the DRB

to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected PBMC. Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the DRB and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 and Table 9.1.4 for Safety Follow-up.

Table 9.1.5, dealing with therapeutic corticosteroid use in the event of elevated LFTs, is discussed in Section 9.4.8.2.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	Smart Rescreening[i] (Day -28 to Day -1)	Baseline (Day -7 to Day -1)[h]	
Informed consent	X			
Medical History	X			
Physical Examination[a]	X		X	X
Height and Weight[a]	X			
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, coagulation screen, and CRP[e]	X	X	X	
Urine Tests[e]	X	X	X	
Liver Function Tests[e]	X	X	X	
PBMC collection for CTL baseline			X	
Von Willebrand Factor Antigen (VWF:Ag)	X			
Direct Thrombin Test			X	
PCR of vector DNA in blood, saliva, urine, semen, and stools			X	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

[b] Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). Sample collection on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing on these samples is optional.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, LFTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion must be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up (Week 1-16)

Assessment	Follow-Up After BMN 270 Administration – Weeks*																
	Week 1																
	D2	D4	D8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical examination[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X		X				X
Urine Tests[b]						X				X				X				X
Liver Function Tests (local)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Function Tests (central)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer						X				X				X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X	X	X			X		X		X		X		X		X		X
Exploratory biomarker assessments[f]						X				X				X				X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C																		X[g]
PBMC collection			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Von Willebrand Factor Antigen (VWF:Ag)						X				X				X				X
Direct Thrombin test			X												X			

[*] Visit windows are ± 48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then until at least 3 consecutive negative results are obtained. Collection and testing of semen samples must continue through Week 12, even if 3 consecutive negative samples in that compartment have already been recorded.

[f] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing on these samples is optional.

[g] Testing for reactivation of hepatitis B and hepatitis C at Week 16 should be performed only in subjects who have not received therapeutic corticosteroids prior to Week 16; subjects who have received therapeutic corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.5.

Table 9.1.3: Schedule of Events – Safety Follow-Up (Week 17-32)

Assessment	Follow-Up After BMN 270 Administration – Weeks*															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight										X						
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X				X				X				X
Urine Tests[b]				X				X				X				X
Liver Function Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Function Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer				X								X				
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X				X				X				X
Exploratory biomarker assessments[f]				X				X								
Haemo-QoL-A QoL assessment												X				
AAV5 antibody titer				X				X				X				X
PBMC collection	X	X	X	X		X		X		X		X		X		X
Von Willebrand Factor Antigen (VWF:Ag)				X				X				X				X
Direct Thrombin test										X						

* Visit windows are ± 48 hours

[a] Brief physical examination should be done at all weekly visits. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing on these samples is optional.

Table 9.1.4: Schedule of Events – Safety Follow-Up

Assessment	Year 1 – Weeks*												Years 2-5	ETV
	33	34	35	36	38	40	42	44	46	48	50	52	Q3M	
Physical examination[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight[a]												X	X[a]	
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X	X	X
Urine Tests[b]				X		X		X		X		X	X	X
Liver Function Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Function Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AAV5 antibody titer				X		X		X		X		X	X	X
FVIII antibody titer				X				X				X	X	X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)		X		X				X				X	X	X
Von Willebrand Factor Antigen (VWF:Ag)				X		X		X		X		X	X	X
Direct Thrombin Test					X							X	X	X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X		X		X		X		X		X
Haemo-QoL-A QoL assessment												X	X[f]	X

* Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52 and ± 2 weeks for visits in Years 2-5.

[a] Complete physical examination should be performed at Week 52 and every 52 weeks thereafter; brief physical examination may be performed at other study visits. Refer to Section 9.7.8.5. During Years 2-5, weight should be performed every 6 months.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

[f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).

Table 9.1.5: Schedule of Events – Therapeutic Corticosteroids

	Steroid Treatment Period[d]											Post-Steroid Period[c]				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11[b]**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 13**
Therapeutic corticosteroids (dose in mg/day) [a]	60 mg	60 mg	40 mg	40 mg	30 mg	30 mg	20 mg	20 mg	15 mg	10 mg	5 mg					
FVIII activity testing												X	X	X	X	
Liver function testing												X	X	X	X	
Hepatitis B testing						X						X				X
HCV Viral Load						X						X				X

[a] Therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor.

[b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

[c] After discontinuation of corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. If these assessments are already being done as part of normal study follow-up, they do not need to be duplicated.

[d] Regardless of the assessments listed in the Schedule of Assessments (Table 9.1.2, Table 9.1.3, or Table 9.1.4), subjects initiated on corticosteroids will only be required to have laboratory evaluations on a weekly basis.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4.
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- Any ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270, in the absence of a definitive alternate etiology for the increase
- The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion
- The detection of AAV vector DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than 52 weeks after BMN 270 infusion, as discussed in Section 9.7.8.6
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt:

- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days

9.3.4 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.5 Duration of Subject Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical examination performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (e.g. the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be

discharged from the clinic unless toxicity has been observed in which case the stay in the clinic may be extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

For Cohorts 1 to 3, approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1)

at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in that cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design for Cohorts 1-3

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents

Medications which are predominately metabolized by the liver (e.g., acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LFTs (at local labs, once per week for Weeks 1-36, then once every 2 weeks from Week 37-52). LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

9.4.8.2.1 Therapeutic Corticosteroids

In general, therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator.

Reports of raised LFTs (defined as ALT ≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

Following initiation or completion of therapeutic corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

Treatment with prednisolone will be initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.5).

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

Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug

appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1 through Table 9.1.4) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to expression of FVIII at 5 IU/dL or above, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes (Den Ujil, 2011, Haemophilia).

The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic FXa assay)
- FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time)

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

The FVIII activity level in both assays and the number of subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.

FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4, but will be used in conjunction with local lab LFT assessments to monitor subject safety and need for initiation of therapeutic corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage APTT and/or chromogenic FXa assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum

concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.6 Exploratory Assessments

Blood samples will be collected from subjects at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing is optional.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed.

On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with samples collected specifically for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	Haematology	Urine Tests	Other
Albumin	Haemoglobin	Appearance	CRP
BUN	Haematocrit	Color	
Calcium	WBC count	pH	**Coagulation Screen including:**
Chloride	RBC count	Specific gravity	APTT
Total cholesterol	Platelet count	Ketones	PT/INR
CO_2	Differential cell count	Protein	TT
CPK		Glucose	
Creatinine		Bilirubin	
Glucose		Nitrite	
Phosphorus		Urobilinogen	
Potassium		Haemoglobin	
Total protein			
Sodium			
Uric Acid			

BUN, blood urea nitrogen; CO_2, carbon dioxide; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

9.7.8.3 Liver Function and Hepatitis Testing

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active Hepatitis B or Hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing Hepatitis B or Hepatitis C infection is exclusionary. Subjects who have cleared a Hepatitis B infection or are seronegative do not need to receive the Hepatitis B vaccination.

Subjects will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.

A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopoenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

Liver function tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4. At each time point, the following LFTs should be assessed:

Table 9.7.8.3.1: Liver Function Tests

Liver Function Tests			
Alkaline Phosphatase	AST (SGOT)	Total Bilirubin	LDH
ALT (SGPT)	Direct Bilirubin	GGT	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase

Patients with ALT > 1.5 ULN during the study may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

9.7.8.4 HIV Testing

HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.

9.7.8.5 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and every 52 weeks thereafter; at other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems.

A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, and gastrointestinal assessments.

Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

9.7.8.6 Vector Shedding

Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable

after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid but not in motile sperm (Manno, 2006, Nat.Med.). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016, Haemophilia).

During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of vector shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the subject is a haemophiliac) should not be recorded as AEs but will instead be captured in subject diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of major trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening

  Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (e.g. anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of liver enzymes (ALT) that triggers an initiation or modification of corticosteroid treatment

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.2. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE (or SAE, as appropriate) eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. Events that change in severity necessitate the recording of an additional AE. AEs that do not have a change in severity should be recorded only once on the eCRF.

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death

is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site

becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the

investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax: PI
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI, MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic FXa assay and the one-stage APTT assay are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LFTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, Hepatitis B, Hepatitis C, and HIV.
- Complete Physical Examination
- Height and weight
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic FXa (plasma)
    - Baseline hFVIII activity level – one-stage APTT assay
    - hFVIII coagulation activity exploratory assay
    - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
    - hFVIII total antibody assay
    - hFVIII antigen (ELISA)

- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Screen for Hepatitis B, Hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)
- Von Willebrand Factor Antigen (VWF:Ag)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)

- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)
- PBMC collection for CTL baseline
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- BMN 270 Infusion
- Vital Signs

- Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Once per week (Weeks 1 through 16)

The following procedures will be performed at one visit per week from Weeks 1 through 16:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
  - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- Samples for FVIII Assays – central laboratory assessment
  - FVIII activity level (one-stage APTT)
  - FVIII activity level (chromogenic FXa assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
  - Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
  - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- PBMC collection

12.5.2 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only) – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – central laboratory assessment
- Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – local laboratory assessment
- FVIII activity level (Day 4 only) – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)

12.5.3 Week 1 – Day 8

On Day 8, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Direct Thrombin test
- Haemo-QoL-A QoL assessment

12.5.4 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) (not assessed at Week 14)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2)
    - Collection to occur until at least 3 consecutive negative results are obtained. Semen samples should continue to be collected and tested through Week 12, even if 3 consecutive negative results in that compartment have been recorded prior to that timepoint.

12.5.5 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedures will be performed:

- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- FVIII antibody titer
- Exploratory biomarker assessments

12.5.6 Week 16

At Week 16, the following procedures will be performed:

- Test for Hepatitis B and Hepatitis C reactivation
    - Subjects will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.

12.5.7 Weeks 8 and 16

At Weeks 8 and 16, the following procedures will be performed:

- AAV5 antibody titer

12.5.8 Weeks 2, 3, 4, and 16

At Weeks 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.5.9 Week 13

At Week 13, the following procedure will be performed:

- Direct Thrombin test

12.6 Safety Follow-Up – Weeks 17-36

After the Post-Infusion Follow-Up visits are complete, subjects will return to the study site for Safety Follow-Up visits from Weeks 17 through Week 36 (± 48 hours), when the following procedures will be completed:

12.6.1 Once per week (Weeks 17 through 36)

Once per week from Week 17 through Week 36, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)

- FVIII activity level (chromogenic FXa assay)

12.6.2 Once per week (Weeks 17 through 20)

Once per week from Week 17 through Week 20, the following procedures will be performed:

- PBMC collection

12.6.3 Every 2 weeks (Weeks 21 through 36)

Every 2 weeks (Weeks 22, 24, 26, 28, 30, 32, 34, and 36), the following procedures will be performed:

- PBMC collection

12.6.4 Every 4 Weeks

Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed:

- Exploratory biomarker assessments (Weeks 20 and 24 only)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- AAV5 antibody titer
- PCR of vector DNA in blood, saliva, urine, semen, and stools
  - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.5 Every 8 Weeks

Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed:

- FVIII antibody titer

12.6.6 Week 26

At Week 26, the following procedure will be performed:

- Direct Thrombin test
- Weight

12.6.7 Week 28

At Week 28, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Weeks 37-52

Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:

12.7.1 Once per visit

At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

12.7.2 Every 4 Weeks

Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.7.3 Every 8 Weeks

Every 8 weeks (Weeks 44 and 52), the following procedure will be performed:

- PBMC collection
- FVIII antibody titer

12.7.4 Week 38 and 52

At Week 38 and Week 52, the following procedure will be performed:

- Direct Thrombin test

12.7.5 Week 52

At Week 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment
- Weight

12.8 Safety Follow-Up – Years 2-5

During Years 2-5 of Safety Follow-up, subjects will be assessed every 3 months (± 2 weeks). At these times, the following procedures will be completed:

12.8.1 Every Visit

Every 3 months (± 2 weeks), the following procedures will be performed:

- Physical examination
    - Complete Physical Examination will be performed every 52 weeks; Brief Physical Examinations may be performed at other visits.

- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (FXa chromogenic assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test

12.8.2 Every Other Visit (Every 6 Months)

Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedure will be performed:

- Haemo-QoL-A QOL assessment
- Weight

12.9 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 260 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical examination
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
- FVIII activity level – local laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
- FVIII Assays – central laboratory assessment
    - FVIII activity level (one-stage APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Haemo-QoL-A QOL assessment

12.10 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 260). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay as measured by the central laboratory at 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.4 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma

profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.5 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.6 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.7 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon

completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB/IEC/REB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201, Amendment 5

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

DocuSigned by:
PI
14-Feb-2017 | 8:34 AM PST

Medical Monitor Signature Date

Signer Name: PI
Signing Reason: I approve this document
Signing Time: PI
50FE2C0CB1C24F7C96F74CC498E3FB2F

Printed name: PI, MD, PI, Clinical Sciences

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See pages 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Text Revisions	Rationale
2/Synopsis (Criteria for Evaluation)	No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LFTs (at local labs, ~~twice~~ once per week for Weeks 1-~~20~~36, ~~then once per week from Week 21-36, and~~ then once every 2 weeks from Week 37-52) during Year 1. LFTs will be monitored every three months for up to 5 years post-dose in the safety extension; the frequency and duration of LFT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.	1
7/Introduction	Gene therapy offers the potential of ~~a cure through~~ disease-modifying therapy by continuous endogenous production of human FVIII following a single administration of vector.	5
Tables 9.1.1, 9.1.2, 9.1.3, 9.1.4	*Schedules of Assessments and footnotes were updated to be consistent with other changes made elsewhere in the protocol.*	1, 2, 3, 5
9.4.8.2/Glucocorticoid Treatment of Elevated Hepatic Transaminases	During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LFTs (at local labs, ~~twice~~ once per week for Weeks 1-36~~20~~, ~~then once per week from Week 21-36, and~~ then once every 2 weeks from Week 37-52). LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.	1
9.7.6/Exploratory ~~Biomarker~~ Assessments	On an exploratory basis, samples may be fractionated prior to shedding analysis in order to better characterize the presence and location of vector DNA and/or vector capsid within each matrix. The fractionation may be performed with samples collected specifically for shedding analysis (saliva, blood, semen, urine, faeces), or by using exploratory samples, such as plasma, PBMCs, and red blood cells, collected under the study protocol.	4
9.7.8.3/Liver Function and Hepatitis Testing	Patients with ~~persistent~~ ALT > 1.5 ULN during the study ~~and no other etiology identified~~ may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.	5

Section No./Title	Text Revisions	Rationale
9.7.8.6/Vector Shedding	Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Testing of semen will continue through Week 12, even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint.	3
10.4.1.1/Diagnosis versus Signs and Symptoms	Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE (or SAE, as appropriate) eCRF, replacing the original entries where appropriate.	5
10.4.1.9/Pregnancy	Pregnancy in a partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner (with that partner's consent) through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials.	5
12.5.1/Once per Week (Weeks 1-16)	The following procedures will be performed at one visit per week from Weeks 1 through 16: • Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment o LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII activity level – local laboratory assessment o FVIII activity level (one-stage APTT) o FVIII activity level (chromogenic FXa assay) • PMBC collection	1, 2
~~12.5.2/Twice per Week (Weeks 1-16)~~	~~The following procedures will be performed twice per week from Weeks 1 through 16:~~ ~~• Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment~~ ~~o Local assessment of liver function tests should be performed on Day 8 during Week 1, and then 2x/week from Week 2 through Week 16~~ ~~o LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.~~	1

Section No./Title	Text Revisions	Rationale
	~~• FVIII activity level – local laboratory assessment~~ ~~○ FVIII activity level (APTT)~~ ~~○ FVIII activity level (chromogenic FXa assay)~~ ~~▪ Local assessment of FVIII activity level should be performed on Day 8 during Week 1, and then 2x/week from Week 2 through Week 16~~	
12.5.4/Every 2 Weeks	Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed: • PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2) ○ Collection to occur until at least 3 consecutive negative results are obtained. Semen samples should continue to be collected and tested through Week 12, even if 3 consecutive negative results in that compartment have been recorded prior to that timepoint.	3
12.5.5/Weeks 4, 8, 12, and 16	At Weeks 4, 8, 12, and 16, the following procedures will be performed: ~~• PBMC collection~~	2
12.6.1/Once per week (Weeks 17 through 36)	Once per week from Week 17 through Week 36, the following procedures will be performed: • Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII activity level – local laboratory assessment ○ FVIII activity level (one-stage APTT) ○ FVIII activity level (chromogenic FXa assay)	1
12.6.2/~~Twice~~ Once per Week (Weeks 17 through 20)	~~Twice~~ Once per week from Week 17 through Week 20, the following procedures will be performed: • PBMC collection ~~• Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment~~	1, 2

Section No./Title	Text Revisions	Rationale
	~~○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.~~ ~~• FVIII activity level – local laboratory assessment~~ ~~○ FVIII activity level (APTT)~~ ~~○ FVIII activity level (chromogenic FXa assay)~~	
12.6.3/~~Once per week~~ Every Two Weeks (Weeks 21 through 36)	~~Once per week from Week 21 through Week 36~~ Every two weeks (Weeks 22, 24, 26, 28, 30, 32, 34, and 36), the following procedures will be performed: ~~• Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment~~ ~~○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.~~ ~~• FVIII activity level – local laboratory assessment~~ ~~○ FVIII activity level (APTT)~~ ~~○ FVIII activity level (chromogenic FXa assay)~~ • PBMC collection	1, 2
12.6.4/Every 4 Weeks	Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed: ~~• PBMC collection~~	2
References	Heller CG & Clermont Y. Kinetics of the germinal epithelium in man. Recent Prog Horm Res. 1964;20:545-571. Heller CG, Heller GV, Rowley MJ. Human spermatogenesis: an estimate of the duration of each cell association and each cell type. Excerpta Med Int Congr Ser. 1969;184:1012-1018.	3

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI , MD PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Subject Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015
Date of Amendment 4:	02 September 2016

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 4

Date: 2 September 2016

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 4 to the BMN 270-201 protocol is provided below.

1. The requirement for prophylactic corticosteroids has been removed and the threshold for starting therapeutic corticosteroids has been changed from alanine aminotransferase (ALT) ≥ 1.5x baseline value to ≥ 1.5x upper limit of normal (ULN).

Rationale: Previous FIX gene-therapy clinical studies have demonstrated an association between increases in ALT and declines in FIX expression, which are thought to be due to a cell-mediated immune response to transduced hepatocytes (Manno, 2006, Nat.Med.) Mingozzi, 2013, Blood; (Nathwani, 2014, N.Engl.J.Med.); (Nathwani, 2011, Mol.Ther.). In one study (Nathwani, 2014, N.Engl.J.Med.); (Nathwani, 2011, Mol.Ther.), partial rescue of FIX expression was observed with the initiation of corticosteroids. Based on these results, as a conservative measure to preserve FVIII activity, the BMN 270-201 protocol set the threshold for initiating a therapeutic course of corticosteroids at ≥ 1.5x baseline. Further, following this event in the first subject, all subsequent subjects were to receive prophylactic corticosteroid therapy. To date, however, in clinical study 270-201, mild asymptomatic elevations in ALT largely near or slightly above the ULN have been observed following dosing, but in general, have not been associated with a concomitant drop in FVIII expression. These observations are consistent with preliminary clinical trial data reported from recent AAV-FIX gene therapy studies (George, 2016, Haemophilia; Miesbach, 2016, Haemophilia), which demonstrate that subjects with mild elevations in ALT did not have associated concomitant loss in FIX activity; in one study (George, 2016, Haemophilia), subjects did not require corticosteroid dosing (no hepatic transaminases >1.5x ULN). Given the fact that elevations in ALT values of 1.5x baseline could represent normal fluctuations in liver enzymes (Watkins, 2006, JAMA), the threshold for initiating corticosteroids is being amended to ≥ 1.5x the ULN. Adjusting the threshold to ≥ 1.5x ULN will avoid unnecessary prolonged exposure to corticosteroids at lower levels of ALT elevation. Given the lack of association between changes in liver enzymes and FVIII activity, the 270-201 protocol has been amended to provide increased flexibility and clinical discretion and to recommend initiation of corticosteroids if there is a rise in ALT greater than 1.5x ULN or in consultation

between the investigator and the medical monitor based on the pattern of FVIII activity and/or changes in liver enzymes as well as an ongoing review of efficacy and safety data from subjects on-study to date.

To maintain adequate surveillance of hepatocyte injury and FVIII expression, liver enzymes and FVIII levels will be monitored at regular intervals (at local labs, twice per week for Weeks 1-20, then once per week from Week 21-36, and then once every 2 weeks from Week 37-52) to ensure that any elevations in ALT and decreases in FVIII are observed early which may support intervention with corticosteroids. The monitoring schedule and corticosteroid regimen may be adjusted as needed based on individual subject data upon discussion between the investigator and the medical monitor.

2. An additional cohort of subjects has been added. For Cohort 4, 3 subjects will be enrolled at the dose of 4E13 vector genomes (vg) per kilogram. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

Rationale: In order to fully understand the dose-response relationship of BMN 270, an additional cohort of subjects will be enrolled at a dose of 4E13 vg/kg, a dose level which is intermediate between the high (6E13 vg/kg) and mid (2E13 vg/kg) dose levels. A dose level of 4E13 vg/kg was chosen in order to target FVIII levels within the normal range, which is expected to prevent the need for exogenous FVIII administration under most circumstances, such as peri-surgically or following serious trauma (White, 2001, Thromb Haemost). The current dose of 6E13 vg/kg has resulted in FVIII levels above the upper limit of normal in 2 of 7 treated patients, which suggests that a lower dose could provide more optimal FVIII expression and activity levels. The 4E13 dose is expected to be effective based on clinical experience to date in 270-201 as improvements in FVIII activity and an acceptable safety profile were observed in both Cohort 2 (2E13) and Cohort 3 (6E13).

3. The enrollment stopping criterion related to serum ALT levels has been changed from a 5-fold increase from baseline after BMN 270 administration to:

   ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270 in the absence of a definitive alternate etiology for the increase.

Rationale: Given that 270-201 is a first in human study, a 5-fold ALT increase from baseline was considered, at study initiation, to be an appropriately conservative approach to enhancing patient safety. Clinical experience to date with BMN 270 demonstrates mild asymptomatic increases in ALT from baseline values, which have not to date exceeded Common Terminology Criteria for Adverse Events (CTCAE) grade 1 toxicity level (up to 3x ULN). Most ALT increases from baseline were within the normal range or slightly

greater than the ULN. Given that the fluctuations in liver enzymes observed above baseline values in treated subjects are potentially in-line with normal variations found in healthy volunteers (Watkins, 2006, JAMA) and as safety data to date generated with AAV vectors with tropism to the liver (Nathwani, 2014, N.Engl.J.Med.); Pasi, 2016, Haemophilia; Monahan, 2015, J. Thromb Haemostasis; George, 2016, Haemophilia; Miesbach, 2016, Haemophilia; (Manno, 2006, Nat.Med.); (Nathwani, 2011, Mol.Ther.), have found reversible and, in most cases, mild elevations in ALT without signs of significant hepatic injury, the ALT enrollment stopping criteria has been modified to 5x the ULN with a duration of at least 2 consecutive weeks. This criterion is in line with FDA guidance on drug-induced liver injury. To support patient safety, monitoring of liver enzymes will be performed frequently and at regular intervals (at local labs, twice per week for Weeks 1-20, then once per week from Week 21-36, and then once every 2 weeks from Week 37-52) to ensure that any elevations in ALT are observed early, with repeat testing to confirm the abnormality. In addition, when considering whether to dose subsequent subjects following an elevation in ALT in any treated subject, other factors that may have directly caused the rise in ALT (eg, excessive alcohol use or ALT rise from non-liver origin such as from excessive physical strain) will be considered to fully evaluate the etiology of the increase.

4. Frequency of monitoring of vector shedding assessment (by polymerase chain reaction [PCR]) during the Post-Infusion Follow-Up period (Weeks 1 to 16) has been reduced to every other week between Week 4 and Week 16.

Rationale: In the Post-Infusion Follow-Up period, collection every 2 weeks after Week 4 is sufficient, as clinical study data generated to date demonstrates that the earliest biofluid to clear is urine, but not earlier than Week 4. A visit every week presents an unnecessary burden on subjects given the slow clearance in most fluids.

5. Frequency of monitoring of the vector shedding assessment (by PCR) in the Safety Follow-Up period (after Week 16) has been changed to include assessments every 4 weeks between Weeks 20 and 52.

Rationale: More frequent testing after week 20 is needed in order to better characterize the time point at which vector shedding has ceased. Per protocol, testing of vector shedding in any fluid compartment can be stopped after 3 consecutive negative tests. In the case of vector shedding in the semen, more frequent testing will avoid prolonged and unnecessary contraception use.

6. The enrollment stopping criterion around vector shedding has been modified. Enrollment in the study will be put on halt if there is persistent detection (defined as 3 consecutive positive samples) of AAV vector DNA in the semen of a participant more than 52 weeks after BMN 270 administration.

Rationale: The original enrollment stopping criterion related to vector shedding had set a threshold of 26 weeks if detected in the semen, based on expected clearance from AAV clinical studies (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid for 16 weeks, but not in motile sperm (Manno, 2006, Nat.Med.). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV5-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016, Haemophilia). Similarly, clinical study data from 270-201 has demonstrated that subjects show a residual, but progressively declining level of vector DNA in the semen with very low copies identified past week 26 in one subject to date (2 copies/5µL qPCR sample at week 28 with 2 prior negative values). The slow clearance from the semen mirrors clearance from the blood compartment and as the semen values are declining with a very low copy level at week 28, shedding in semen is expected to clear by week 40. The modified enrollment stopping criterion is deemed appropriate to allow for complete clearance from the semen while controlling the potential risk to patient safety that may be posed by the continued persistence of the vector DNA in semen. Subjects will continue effective contraception use until at least 3 consecutive semen samples are negative for vector DNA.

7. Stopping criterion regarding Grade 2 related events has been changed. The occurrence of a Grade 2 related adverse event persisting for at least 7 days will now trigger a Data Review Board (DRB) review of safety data to determine whether an enrollment halt is warranted, rather than triggering an automatic enrollment halt.

Rationale: The original stopping criterion was set very conservatively, in the absence of any clinical data for BMN 270 at the start of the first-in-human study. Given the safety profile observed to date in 9 subjects dosed with BMN 270, an automatic enrollment halt based on a related Grade 2 event of 7 days duration is not warranted. To date, no subject has experienced a Grade 2 related event lasting 7 days or more. As such, a prolonged Grade 2 event assessed by the investigator as related to BMN 270 will trigger a DRB review of study

safety; the DRB will be consulted to evaluate the event as well as the totality of safety data and may choose to halt enrollment or not based upon the results of that review.

8. Testing of von Willebrand factor Antigen (VWF:Ag) has been added as an assessment (at Screening and the same time points that FVIII activity is measured during the study).

Rationale: As fluctuations in FVIII levels are commonly observed and may be related to changes in VWF levels (e.g. increase in VWF secondary to exercise), following VWF levels at regular intervals over the course of the study will enable a more complete assessment of any on-study changes in FVIII levels.

9. Testing of creatine phosphokinase (CPK) has been added to routine blood chemistry assessments during the study. CPK testing may be performed at other intervals based on PI assessment.

Rationale: CPK will allow for a more complete assessment of elevations in ALT and/or alanine aminotransferase (AST) by potentially differentiating between muscle and liver as the source of the enzymes. An elevation of CPK may indicate muscle injury and not hepatic injury.

10. An exploratory Direct Thrombin assay has been added at Baseline, Week 1, and every 3 months thereafter.

Rationale: To better understand the relationship between FVIII levels and the downstream clotting cascade following administration of BMN 270, a direct thrombin assay will be performed.

11. The identity of the medical monitor has been changed.
12. Minor edits for clarity and consistency have been incorporated.

Specific changes included in this amendment, including the Synopsis, since Amendment 3 (approved 6 November 2015) are outlined in Section 24.

2 SYNOPSIS

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TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life

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by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 1).

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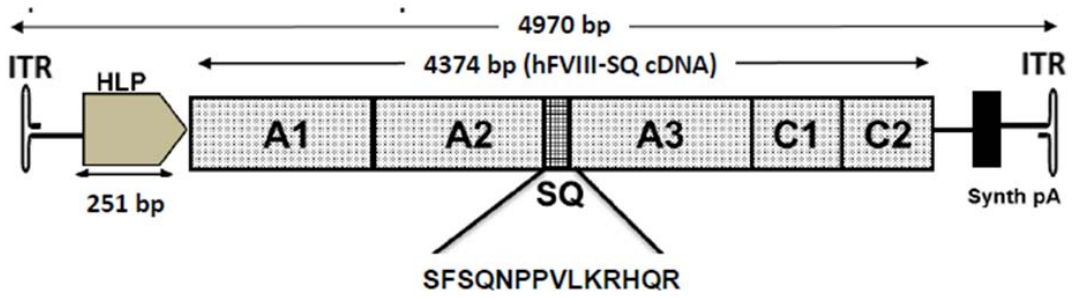
Figure 1. hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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

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during the study

STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)

Cohort 2: 2E13 vg per kilogram, iv

Cohort 3: 6E13 vg per kilogram, iv

Cohort 4: 4E13 vg per kilogram, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Cohorts 1-3

The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the aPTT and chromogenic assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

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Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.
Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

NUMBER OF SUBJECTS PLANNED:

Up to 15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

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1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
    - ALT (alanine transaminase) to 3 times the upper limit of normal;
    - Bilirubin above 3 times the upper limit of normal;
    - Alkaline phosphatase above 3 times the upper limit of normal; or
    - INR (international normalized ratio) ≥ 1.4
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A

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7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine $\geq$ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous infusion.

CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)

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- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver function tests (LFTs, including ALT, AST, GGT, LDH, bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. Each subject will have comprehensive surveillance monitoring of LFTs (at local labs, twice per week for Weeks 1-20, then once per week from Week 21-36, and then once every 2 weeks from Week 37-52) during Year 1. LFTs will be monitored every three months for up to 5 years post-dose in the safety extension; the frequency and duration of LFT testing may be changed based on discussion between the Medical Monitor and the Investigator and review of subject data.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive

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in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay at 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as vector shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DRB	Data Review Board
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
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
HA	Haemophilia A
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee
IND	Investigational New Drug (application)

INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LFT	liver function test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
ULN	upper limit of normal
vg	vector genomes
VWF:Ag	von Willebrand factor Antigen

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing

eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver function tests (LFTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LFTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman, 2013, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay, 2012, Blood).

Gene therapy offers the potential of a cure through continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2 ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2013, Haemophilia.); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This

nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in

the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani, 2006, Blood). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Engl.J.Med.), (Nathwani, 2014, N.Engl.J.Med.). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola, 2014, J.Hepatol.). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Mannucci, 2001, N.Engl.J.Med.). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. . The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

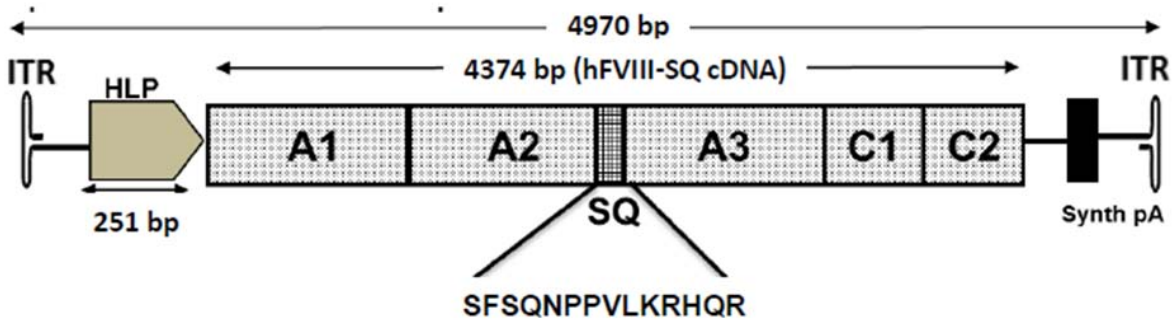
Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011, Haemophilia.). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia.), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci, 2001, N.Engl.J.Med.). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol.Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, 2014, N.Engl.J.Med.). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.), (Bainbridge, 2008, N.Engl.J.Med.), (Maguire, 2009, Lancet); (Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of hFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: hFVIII-SQ Vector Genome Schematic



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan (Nathwani, 2011, Mol.Ther.). Each subject will have a comprehensive surveillance plan that monitors LFTs during the study.

For additional information on safety findings in 270-201, refer to current version of the Investigator's Brochure.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related

changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to four cohorts according to dose level:

- Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
- Cohort 2: 2E13 vg per kilogram, iv
- Cohort 3: 6E13 vg per kilogram, iv
- Cohort 4: 4E13 vg per kilogram, iv

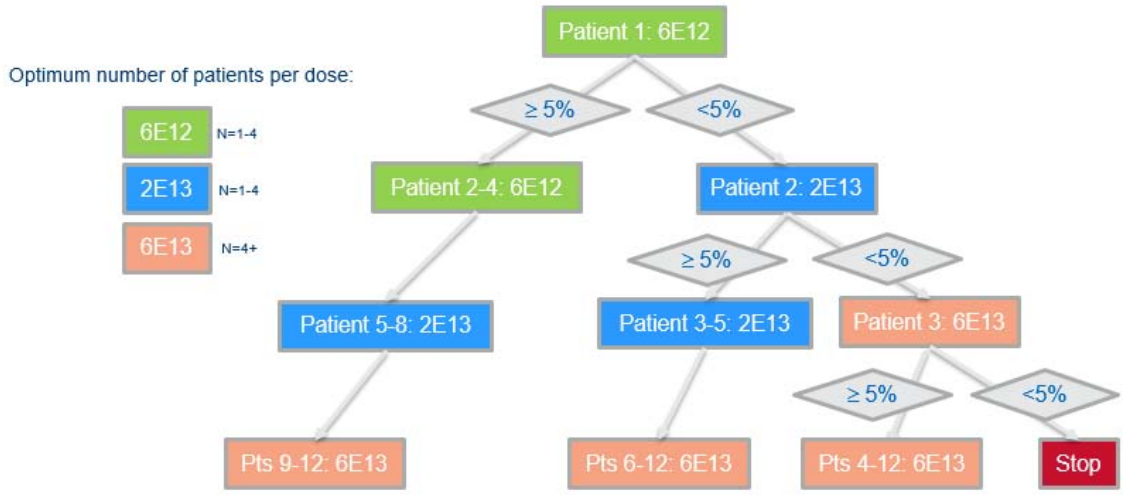
The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in mice.

Cohorts 1-3

The first three cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme for Cohorts 1 to 3



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Cohort 4

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC). Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the Data Review Board (DRB) and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 and Table 9.1.4 for Safety Follow-up.

Table 9.1.5, dealing with therapeutic corticosteroid use in the event of elevated LFTs, is discussed in Section 9.4.8.2.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	**Smart Rescreening[i] (Day -28 to Day -1)**	**Baseline (Day -7 to Day -1)[h]**	
Informed consent	X			
Medical History	X			
Physical Exam[a]	X		X	X
Height and Weight[a]	X			
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, coagulation screen, and CRP[e]	X	X	X	
Urine Tests[e]	X	X	X	
Liver Function Tests[e]	X	X	X	
PBMC collection for CTL baseline			X	
Von Willebrand Factor Antigen (VWF:Ag)	X			
Direct Thrombin Test			X	
PCR of vector DNA in blood, saliva, urine, semen, and stools			X	

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	**Smart Rescreening[i] (Day -28 to Day -1)**	**Baseline (Day -7 to Day -1)[h]**	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

[b] Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). The assessment on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing on these samples is optional.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, LFTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion must be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up (Week 1-16)

Assessment	Follow-Up After BMN 270 Administration – Weeks*																	
	Week 1																	
	D2	D4	D8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical exam[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X		X				X
Urine Tests[b]						X				X				X				X
Liver Function Tests (local)[b]		X	X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
FVIII assays (local)[c]		X	X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
Liver Function Tests (central)[b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer						X				X				X				X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]	X	X	X			X		X		X		X		X		X		X
Exploratory biomarker assessments[f]						X				X				X				X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C																		X[g]
PBMC collection						X				X				X				X
Von Willebrand Factor Antigen (VWF:Ag)						X				X				X				X
Direct Thrombin test			X												X			

[*] Visit windows are ± 48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with persistent ALT > 1.5 ULN during the study and no other etiology identified may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing on these samples is optional.

[g] Testing for reactivation of hepatitis B and hepatitis C at Week 16 should be performed only in subjects who have not received therapeutic corticosteroids prior to Week 16; subjects who have received therapeutic corticosteroids will have hepatitis B and hepatitis C testing at the time points indicated in Table 9.1.5.

Table 9.1.3: Schedule of Events – Safety Follow-Up (Week 17-32)

Assessment	Follow-Up After BMN 270 Administration – Weeks*															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Physical exam[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight										X						
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X				X				X				X
Urine Tests[b]				X				X				X				X
Liver Function Tests (local)[b]	XX	XX	XX	XX	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	XX	XX	XX	XX	X	X	X	X	X	X	X	X	X	X	X	X
Liver Function Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer				X								X				
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X				X				X				X
Exploratory biomarker assessments[f]				X				X								
Haemo-QoL-A QoL assessment												X				
AAV5 antibody titer				X				X				X				X
PBMC collection				X				X				X				X
Von Willebrand Factor Antigen (VWF:Ag)				X				X				X				X
Direct Thrombin test										X						

* Visit windows are ± 48 hours

[a] Brief physical examination should be done at all weekly visits. Refer to Section 9.7.8.5.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with persistent ALT > 1.5 ULN during the study and no other etiology identified may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (APTT and FXa chromogenic assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Collection to occur until at least 3 consecutive negative results are obtained.

[f] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing on these samples is optional.

Table 9.1.4: Schedule of Events – Safety Follow-Up

Assessment	Year 1 – Weeks*												Years 2-5	ETV
	33	34	35	36	38	40	42	44	46	48	50	52	Q3M	
Physical exam[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight[a]												X	X[a]	
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, coagulation screen, and CRP[b]				X		X		X		X		X	X	X
Urine Tests[b]				X		X		X		X		X	X	X
Liver Function Tests (local)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (local)[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver Function Tests (central)[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays (central)[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AAV5 antibody titer				X		X		X		X		X	X	X
FVIII antibody titer				X				X				X	X	X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)				X				X				X	X	X
Von Willebrand Factor Antigen (VWF:Ag)				X		X		X		X		X	X	X
Direct Thrombin Test					X							X	X	X
PCR of vector DNA in blood, saliva, urine, semen, and stools[e]				X		X		X		X		X		X

Haemo-QoL-A QoL assessment												X	X[f]	X

[*] Visit windows are ± 48 hours through Week 36, then ±1 week until Week 52 and ± 2 weeks for visits in Years 2-5.

[a] Complete physical examination should be performed at Week 52 and every 52 weeks thereafter; brief physical exam may be performed at other study visits. Refer to Section 9.7.8.5. During Years 2-5, weight should be performed every 6 months.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included, and to Table 9.7.8.3.1 for liver function tests. LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. Patients with persistent ALT > 1.5 ULN during the study and no other etiology identified may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[d] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved. If FVIII levels are elevated or stable over the course of several weeks, assessment of FVIII activity may be adjusted based on discussion between the Medical Monitor and the Investigator. A d-dimer may be considered under circumstances in which FVIII activity levels are above the normal physiological range and/or if there is a concern for a venous thromboembolism.

[e] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

[f] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).

Table 9.1.5: Schedule of Events – Therapeutic Corticosteroids

	Steroid Treatment Period[d]											Post-Steroid Period[c]				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11[b]**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 13**
Therapeutic corticosteroids (dose in mg/day) [a]	60 mg	60 mg	40 mg	40 mg	30 mg	30 mg	20 mg	20 mg	15 mg	10 mg	5 mg					
FVIII activity testing												X	X	X	X	
Liver function testing												X	X	X	X	
Hepatitis B testing						X						X				X
HCV Viral Load						X						X				X

[a] Therapeutic corticosteroids may be initiated when a subject's ALT value is≥ 1.5x ULN or based on review of FVIII and liver enzyme data after consultation between the Investigator and the Medical Monitor.

[b] Following initiation or completion of steroid regimen, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

[c] After discontinuation of corticosteroids, weekly labs for ALT and FVIII levels will be measured once a week for 4 weeks to ensure stability in values. If these assessments are already being done as part of normal study follow-up, they do not need to be duplicated.

[d] Regardless of the assessments listed in the Schedule of Assessments (Table 9.1.2, Table 9.1.3, or Table 9.1.4), subjects initiated on corticosteroids will only be required to have laboratory evaluations on a weekly basis.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
    - ALT (alanine transaminase) to 3 times the upper limit of normal;
    - Bilirubin above 3 times the upper limit of normal;
    - Alkaline phosphatase above 3 times the upper limit of normal; or
    - INR (international normalized ratio) ≥ 1.4.
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn.

A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- Any ALT elevation > 5x ULN for at least 2 consecutive weeks after administration of BMN 270, in the absence of a definitive alternate etiology for the increase

- The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion
- The detection of AAV vector DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than 52 weeks after BMN 270 infusion, as discussed in Section 9.7.8.5
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt:

- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days

9.3.4 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.5 Duration of Subject Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical exam performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (e.g. the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be

discharged from the clinic unless toxicity has been observed in which case the stay in the clinic may be extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

For Cohorts 1 to 3, approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1)

at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in that cohort, though the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort.

There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design for Cohorts 1-3

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents

Medications which are predominately metabolized by the liver (e.g., acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring of LFTs (at local labs, twice per week for Weeks 1-20, then once per week from Week 21-36, and then once every 2 weeks from Week 37-52). LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.

9.4.8.2.1 Therapeutic Corticosteroids

In general, therapeutic corticosteroids may be initiated when a subject's ALT value is ≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator.

Reports of raised LFTs (defined as ALT ≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

Following initiation or completion of therapeutic corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the

Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression.

Treatment with prednisolone will be initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of 11 weeks) (refer to Table 9.1.5).

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

Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. Hepatitis B status and HCV viral load will be rechecked 6 weeks after the start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study

drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1 through Table 9.1.4) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to expression of FVIII at 5 IU/dL or above, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes (Den Ujil, 2011, Haemophilia).

The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic FXa assay)
- FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time)

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

The FVIII activity level in both assays and the number of subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.

FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4, but will be used in conjunction with local lab LFT assessments to monitor subject safety and need for initiation of therapeutic corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage APTT and/or chromogenic FXa assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may

be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.6 Exploratory Biomarker Assessments

Blood samples will be collected from subjects at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing is optional.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed.

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4. The scheduled clinical laboratory tests

are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	**Haematology**	**Urine Tests**	**Other**
Albumin	Haemoglobin	Appearance	CRP
BUN	Haematocrit	Color	
Calcium	WBC count	pH	**Coagulation Screen including:**
Chloride	RBC count	Specific gravity	APTT
Total cholesterol	Platelet count	Ketones	PT/INR
CO_2	Differential cell count	Protein	TT
CPK		Glucose	
Creatinine		Bilirubin	
Glucose		Nitrite	
Phosphorus		Urobilinogen	
Potassium		Haemoglobin	
Total protein			
Sodium			
Uric Acid			

BUN, blood urea nitrogen; CO_2, carbon dioxide; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

9.7.8.3 Liver Function and Hepatitis Testing

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active Hepatitis B or Hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing Hepatitis B or Hepatitis C infection is exclusionary. Subjects who have cleared a Hepatitis B infection or are seronegative do not need to receive the Hepatitis B vaccination.

Subjects will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.

A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopoenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

Liver function tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4. At each time point, the following LFTs should be assessed:

Table 9.7.8.3.1: Liver Function Tests

Liver Function Tests			
Alkaline Phosphatase	AST (SGOT)	Total Bilirubin	LDH
ALT (SGPT)	Direct Bilirubin	GGT	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase

Patients with persistent ALT > 1.5 ULN during the study and no other etiology identified may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.

9.7.8.4 HIV Testing

HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.

9.7.8.5 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and every 52 weeks thereafter; at other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems.

A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, and gastrointestinal assessments.

Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.

9.7.8.6 Vector Shedding

Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable

after 40 days in blood, saliva, urine or stool, but in one study was detected in the seminal fluid but not in motile sperm (Manno, 2006, Nat.Med.). In these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016, Haemophilia).

During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of vector shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the subject is a haemophiliac) should not be recorded as AEs but will instead be captured in subject diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of major trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening

  Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (e.g. anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of liver enzymes (ALT) that triggers an initiation or modification of corticosteroid treatment

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.2. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. Events that change in severity necessitate the recording of an additional AE. AEs that do not have a change in severity should be recorded only once on the eCRF.

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death

is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site

becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the

investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax: PI
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI, MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic FXa assay and the APTT assays are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LFTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, Hepatitis B, Hepatitis C, and HIV.
- Complete Physical Exam
- Height and weight
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic FXa (plasma)
    - Baseline hFVIII activity level – one-stage APTT assay
    - hFVIII coagulation activity exploratory assay
    - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
    - hFVIII total antibody assay
    - hFVIII antigen (ELISA)

- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Screen for Hepatitis B, Hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)
- Von Willebrand Factor Antigen (VWF:Ag)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)

- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1)
- PBMC collection for CTL baseline
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- BMN 270 Infusion
- Vital Signs

- Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Once per week (Weeks 1 through 16)

The following procedures will be performed at one visit per week from Weeks 1 through 16:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- Samples for FVIII Assays – central laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
    - Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16

12.5.2 Twice per week (Weeks 1 through 16)

The following procedures will be performed twice per week from Weeks 1 through 16:

- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - Local assessment of liver function tests should be performed on Day 8 during Week 1, and then 2x/week from Week 2 through Week 16
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
        - Local assessment of FVIII activity level should be performed on Day 8 during Week 1, and then 2x/week from Week 2 through Week 16

12.5.3 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only) – central laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – central laboratory assessment
- Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – local laboratory assessment
- FVIII activity level (Day 4 only) – local laboratory assessment
    - FVIII activity level (APTT)

- FVIII activity level (chromogenic FXa assay)

12.5.4 Week 1 – Day 8

On Day 8, the following procedures will be performed:

- PCR of vector DNA in blood, saliva, urine, semen, and stools
- Direct Thrombin test
- Haemo-QoL-A QoL assessment

12.5.5 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) (not assessed at Week 14)
- PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2)
    - Collection to occur until at least 3 consecutive negative results are obtained

12.5.6 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedure will be performed:

- PBMC collection
- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- FVIII antibody titer
- Exploratory biomarker assessments

12.5.7 Week 16

At Week 16, the following procedure will be performed:

- Test for Hepatitis B and Hepatitis C reactivation
    - Subjects will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.

12.5.8 Weeks 8 and 16

At Weeks 8 and 16, the following procedures will be performed:

- AAV5 antibody titer

12.5.9 Weeks 2, 3, 4, and 16

At Weeks 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.5.10Week 13

At Week 13, the following procedure will be performed:

- Direct Thrombin test

12.6 Safety Follow-Up – Weeks 17-36

After the Post-Infusion Follow-Up visits are complete, subjects will return to the study site for Safety Follow-Up visits from Weeks 17 through Week 36 (± 48 hours), when the following procedures will be completed:

12.6.1 Once per week (Weeks 17 through 36)

Once per week from Week 17 through Week 36, the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

12.6.2 Twice per week (Weeks 17 through 20)

Twice per week from Week 17 through Week 20, the following procedures will be performed:

- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)

12.6.3 Once per week (Weeks 21 through 36)

Once per week from Week 21 through Week 36, the following procedures will be performed:

- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)

12.6.4 Every 4 Weeks

Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed:

- Exploratory biomarker assessments (Weeks 20 and 24 only)
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- VWF:Ag
- AAV5 antibody titer
- PBMC collection
- PCR of vector DNA in blood, saliva, urine, semen, and stools

- Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.5 Every 8 Weeks

Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed:

- FVIII antibody titer

12.6.6 Week 26

At Week 26, the following procedure will be performed:

- Direct Thrombin test
- Weight

12.6.7 Weeks 28

At Week 28, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Weeks 37-52

Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:

12.7.1 Once per visit

At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment

- LFTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data.

- FVIII Assays – central laboratory assessment
  - FVIII activity level (APTT)
  - FVIII activity level (chromogenic FXa assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

12.7.2 Every 4 Weeks

Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed:

- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- AAV5 antibody titer
- VWF:Ag
- PCR of vector DNA in blood, saliva, urine, semen, and stools
  - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).

12.7.3 Every 8 Weeks

Every 8 weeks (Weeks 44 and 52), the following procedure will be performed:

- PBMC collection
- FVIII antibody titer

12.7.4 Week 38 and 52

At Week 38 and Week 52, the following procedure will be performed:

- Direct Thrombin test

12.7.5 Week 52

At Week 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment
- Weight

12.8 Safety Follow-Up – Years 2-5

During Years 2-5 of Safety Follow-up, subjects will be assessed every 3 months (± 2 weeks). At these times, the following procedures will be completed:

12.8.1 Every Visit

Every 3 months (± 2 weeks), the following procedures will be performed:

- Physical exam
    - Complete Physical Exam will be performed every 52 weeks; Brief Physical Exams may be performed at other visits.
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII activity level – local laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
    - LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
- FVIII Assays – central laboratory assessment
    - FVIII activity level (APTT)
    - FVIII activity level (FXa chromogenic assay)

- FVIII coagulation activity exploratory assay
- Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
- FVIII antigen (ELISA)

Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test

12.8.2 Every Other Visit (Every 6 Months)

Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedure will be performed:

- Haemo-QoL-A QOL assessment
- Weight

12.9 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 260 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Urine Tests (refer to Table 9.7.8.2.1)
- Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
- FVIII activity level – local laboratory assessment

  - FVIII activity level (APTT)
  - FVIII activity level (chromogenic FXa assay)
- Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
- FVIII Assays – central laboratory assessment
  - FVIII activity level (APTT)
  - FVIII activity level (chromogenic FXa assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
  - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- VWF:Ag
- Direct Thrombin test
- PCR of vector DNA in blood, saliva, urine, semen, and stools
  - Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Haemo-QoL-A QOL assessment

12.10 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 260). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay as measured by the central laboratory at 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.4 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma

profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.5 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.6 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.7 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon

completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB/IEC/REB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201, Amendment 4

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

DocuSigned by:
PI
PI
Medical Monitor Signature Date
Signer Name: PI
Signing Reason: I approve this document
Signing Time: PI
50FF2C0CB1C24F7C96F74CC498E3FB2F

Printed name: PI, MD, PI, Clinical Sciences

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See pages 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Text Revisions	Rationale
2/Synopsis/Study Rationale	Figure 1. ~~BMN 270 transgene construct, ~ 5.0 kbp~~h FVIII-SQ Vector Genome Schematic [an updated version of Figure 1 was provided in this protocol amendment]	12
2/Synopsis/Objectives	The primary objectives of the study are: • To determine the dose of AAV5-hFVIII-SQ required to achieve ~~expression of~~ 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.	12
2/Synopsis/Study Design & Plan	Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to ~~three~~four cohorts according to dose level: Cohort 1: 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv) Cohort 2: 2E13 vg per kilogram, iv Cohort 3: 6E13 vg per kilogram, iv Cohort 4: 4E13 vg per kilogram, iv The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates. **Cohorts 1-3** The first 3 cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the aPTT and chromogenic assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. ~~The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.~~	2

Section No./Title	Text Revisions	Rationale
	… For the first subject treated in each ~~Cohort~~of the first 3 cohorts, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort~~. Although the optimum design of this study is three cohorts of 4 subjects,~~ though, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals. **Cohort 4** For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort. There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.	
2/Synopsis/Number of Subjects Planned	Up to ~~12~~15 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.	2
2/Synopsis/Diagnosis and All Criteria for Inclusion and Exclusion	Individuals eligible to participate in this study must meet all of the following criteria: 6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.	6
2/Synopsis/Criteria for Evaluation	Safety: No major toxicity is expected based on preclinical studies in mice and monkeys. ~~An asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan.~~ Each subject will have comprehensive surveillance monitoring ~~LFTs~~ of LFTs (at local labs, twice per week for Weeks 1-20, then once ~~a~~ per week from ~~dose to completion of~~ Week ~~6, 3 times per week from Weeks 7 to 12~~21-36, and then once ~~a week from Weeks 13 to 16. If a subject's ALT is found to be greater than 1.5x his baseline~~every 2 weeks from Week 37-52) during ~~these periods, then~~ Year 1. LFTs will be monitored ~~three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then~~ every three months for up to 5 years post-dose in the safety extension; the frequency and duration of LFT testing may be changed ~~if dictated by ongoing safety evaluations~~ based on discussion between the Medical Monitor and the Investigator and review of subject data.	1, 3, 12

Section No./Title	Text Revisions	Rationale
2/Synopsis/Statistical Methods	The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to ~~12~~15 subjects may be dosed in the study…	2
Figure 7.3.1	~~BMN 270 Transgene Construct, - 5.0 kbph~~ FVIII-SQ Vector Genome Schematic [An updated version of Figure 7.3.1 has been provided in this protocol amendment]	12
7.4/Summary of Overall Risks and Benefits	Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan (Nathwani, 2011, Mol.Ther.). Each subject will have a comprehensive surveillance plan that monitors LFTs ~~once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a subject's ALT is found to be greater than 1.5x baseline~~ during ~~these periods, then LFTs will be monitored three times per week or until~~ the ~~ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.~~ study. For additional information on safety findings in 270-201, refer to current version of the Investigator's Brochure.	1, 3, 12
9.1/Overall Study Design and Plan	Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to ~~three~~four cohorts according to dose level: • Cohort 1: 6E12 vector genomes [vg/~~kg~~] per kilogram of body weight, given as a single intravenous dose (iv) • Cohort 2: 2E13 vg/~~kg~~ per kilogram, iv • Cohort 3: 6E13 vg/~~kg~~ per kilogram, iv • Cohort 4: 4E13 vg per kilogram, iv **Cohort 4** For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort. There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive	1, 2, 12

Section No./Title	Text Revisions	Rationale
	nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals. Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC). Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study enrollment halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the Data Review Board (DRB) and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority. A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 and Table 9.1.4 for Safety Follow-up. Table 9.1.5, dealing with ~~steroid prophylaxis~~ therapeutic corticosteroid use in the event of elevated LFTs, is discussed in Section 9.4.8.2.	
Figure 9.1.1	The figure title was updated.	12
Tables 9.1.1, 9.1.2, 9.1.3, 9.1.4, and 9.1.5	All tables and footnotes were updates to reflect the changes in the protocol amendment. These changes are also reflected as changes in Section 12 and are detailed below.	1, 4, 5, 6, 7, 8, 9, 10, 12
9.2/Discussion of Study Design, Including Choice of Control Group	Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. ~~Three~~Four doses of BMN 270 will be evaluated and the dose escalation decision tree for Cohorts 1-3 is summarized in Figure 9.1.1.	2
9.3/Selection of Study Population	Up to ~~12~~15 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.	2

Section No./Title	Text Revisions	Rationale
9.3.1/Inclusion Criteria	Individuals eligible to participate in this study must meet all of the following criteria: 6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment. After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.	6
9.3.3.1/Study Safety Evaluation Criteria	If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1. • ~~A 5-fold increase from baseline in~~ Any ALT elevation > 5x ULN for at least 2 consecutive weeks after ~~BMN 270~~ administration of BMN 270, in the absence of a definitive alternate etiology for the increase • The occurrence of Grade 3 or higher adverse events (excluding ALT elevation) assessed as related to study drug, including liver failure and clinical hepatitis • ~~Grade 2 adverse event assessed as related to study drug that persists for at least 7 days~~ • The ~~persistent~~ detection of ~~the~~ AAV vector ~~genome~~DNA in the semen of a participant in 3 consecutive samples (which are at least 2 weeks apart) more than ~~26~~52 weeks after BMN 270 infusion, as discussed in Section 9.7.8.5 If the following event occurs in a subject in the study who has received BMN 270 infusion, a DRB review and analysis of safety data will be undertaken to determine whether the enrollment into the trial will be put on halt: Grade 2 adverse event assessed as related to study drug that persists for at least 7 days	3, 6, 7, 12
9.4.5/Method of Assigning Subjects to Treatment Groups	Cohorts 1 to 3 are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria. Subjects in Cohort 4 will all be enrolled at a single dose.	2
9.4.6.2/Selection of Dose for Each Subject	For Cohort 4, 3 subjects will be enrolled at 4E13 vg/kg. If FVIII activity in these 3 subjects is ≥ 5% at 8 weeks and if no safety issue is found, an additional 3 subjects may be enrolled in this cohort. There will be an ongoing review of individual patient safety and efficacy data by the medical monitor and the DRB. The adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals. Refer to Figure 9.1.1 for a visual representation of the study design~~.~~ for Cohorts 1-3	2, 12

Section No./Title	Text Revisions	Rationale
9.4.8/Prior and Concomitant Medications	All prescription and over-the-counter medications (including dietary and herbal supplements) taken by a subject for 30 days before Screening will be recorded on the designated CRF.… Medications which are predominately metabolized by the liver (e.g., acetaminophen) and alcohol should, whenever possible, be avoided for the first 52 weeks of the study, and particularly within 48 hours prior to lab work.	12
9.4.8.1/Concomitant Haemophilia Treatments	Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary. In addition, information on FVIII usage by medical history will be collected (if available) from subjects for the 6 month period immediately preceding study enrollment.	12
9.4.8.2/Glucocorticoid Treatment of Elevated Hepatic Transaminases	During the Post-Infusion Follow-up Period and the Safety Follow-Up Period, each subject will have comprehensive surveillance plan monitoring ~~LFTs once a week from dose to completion of Week 6, 3 times~~of LFTs (at local labs, twice per week ~~from~~for Weeks ~~7 to 12~~1-20, then once per week from Week 21-36, and then once ~~a week from Weeks 13 to 16. If~~every 2 weeks from Week 37-52). LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.	1, 3
9.4.8.2.1/Therapeutic Corticosteroids	In general, therapeutic corticosteroids may be initiated when a subject's ALT value is ~~found to be greater than 1.5x his baseline during these periods, then LFTs will be monitored three times per week until the ALT returns to baseline.~~≥ 1.5x ULN, or based on review of FVIII and liver enzyme data after consultation between the Medical Monitor and the Investigator. Reports of ~~abnormal~~raised LFTs (defined as ~~1.5x the subject's baseline~~ ALT ~~level~~≥ 1.5x ULN) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available~~, and~~. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Following initiation or completion of therapeutic corticosteroids, if ALT elevation ≥ 1.5x ULN is reported, steroid management decisions will based on discussions between the Investigator and Medical Monitor. Modification of the steroid regimen may take into consideration possible confounders for the ALT elevation and impact on FVIII expression. Treatment with prednisolone will be initiated ~~immediately~~ at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then 15 mg for the next week, then 10 mg for the next week, then 5 mg for the next week, then stop, for a total treatment of ~~8~~11 weeks~~). Local~~	1, 3, 12

Section No./Title	Text Revisions	Rationale
	~~laboratory results of LFTs will be used~~) (refer to Table 9.1.5~~trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.~~). • ~~If a glucocorticoid treatment is started~~After discontinuation of corticosteroids, weekly labs for ~~a subject at any point of the study, the following steps~~ALT and FVIII levels will be ~~taken:~~ o ~~A prophylactic glucocorticoid treatment will be started for subsequent subjects scheduled to be dosed. Subjects will receive prednisolone 40 mg per day starting at~~ measured once a week ~~3,~~ for ~~a duration of~~ 4 weeks~~, then tapered by 5 mg every two weeks for 8 weeks (until 20 mg/day is reached), then tapered 5 mg per week for 3 weeks, for a total duration of 15 weeks (refer to).~~ ~~Subjects already dosed who have not yet reached Week 17~~ to ensure stability in ~~the study will be evaluated for the use of prophylactic steroids after discussion between the Investigator and the Medical Monitor.~~ ~~Once prophylactic glucocorticoid treatments have been initiated, then all subjects (who have not already passed that period in the study) will have 3x/week LFT assessments during the 6-week period when a liver response is expected based on the liver response in the original subject, if this period is different from the 3x/weekly LFT assessments already planned for Weeks 7-12.~~ ~~Subjects who have already passed that 6-week period in the study will continue to have the regularly scheduled LFT assessments as planned in the protocol~~values. Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. Hepatitis B status and HCV viral load will be rechecked ~~at~~6 weeks after the ~~end~~ start of corticosteroid treatment, and then 1 week and 13 weeks after the completion of corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.	
9.7.2/Primary Efficacy Variables	The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to ~~stable~~ expression of FVIII at 5 IU/dL or above, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes~~,~~ (Den Ujil, 2011, Haemophilia). The following assays (assessed by the central laboratory) will be used to measure the primary efficacy variable: • FVIII activity (chromogenic FXa assay)	1, 12

Section No./Title	Text Revisions	Rationale
	• FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time) … FVIII activity assays will also be performed at the local laboratory at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4, but will be used in conjunction with local lab LFT assessments to monitor subject safety and need for initiation of therapeutic corticosteroid dosing; local laboratory FVIII activity assessments will not be used to assess efficacy or to measure the primary efficacy outcome of the study.	
9.7.6/Exploratory Biomarker Assessments	Blood samples will be collected from subjects at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The exploratory genetic/genomic testing ~~on these exploratory samples~~ is optional. All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed. ~~No additional genetic or genomic testing will be conducted on any sample (other than testing specified in the protocol).~~	12
Table 9.7.8.2.1/Clinical Laboratory Tests	This table has been updated as part of this protocol amendment.	1, 3, 9, 12
9.7.8.3/Liver Function and Hepatitis Testing	**9.7.8.3 Liver Function and Hepatitis Testing ~~at Screening~~** Subjects will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5. ... Liver function tests will be monitored on a regular basis, at the time points indicated in Table 9.1.1, Table 9.1.2, Table 9.1.3, and Table 9.1.4. At each time point, the following LFTs should be assessed: **Table 9.7.8.3.1: Liver Function Tests** [this table has been added as part of this protocol amendment]	1, 3, 12

Section No./Title	Text Revisions	Rationale
	Patients with persistent ALT > 1.5 ULN during the study and no other etiology identified may undergo additional evaluations (e.g., imaging studies including MRI or ultrasound, additional blood tests, and/or liver biopsy) at the discretion of the investigator.	
9.7.8.4/HIV Testing	HIV testing will be performed at Screening. Subjects with documented negative results within the last 30 days prior to screening do not need to be retested.	12
9.7.8.5/Vital Signs, Physical Examinations and Other Observations Related to Safety	A complete physical examination is necessary during Screening/Baseline, at Week 16 and 52 and every 52 weeks thereafter; at other visits, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses…. ~~Weight and h~~Height will be recorded at Screening only. Weight will be recorded at Screening and then every 6 months thereafter.	
9.7.8.6/Vector Shedding	**~~9.7.8.6 Viral~~Vector Shedding** ~~Viral~~Vector shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, including Haemophilia B clinical studies utilizing AAV2 and AAV8, vector was no longer detectable after 40 days in blood, saliva, urine or stool~~. However, vector persisted~~ , but in ~~semen for 16 weeks~~one study was detected in the seminal fluid but not in motile sperm (Manno, 2006, Nat.Med.). In ~~all cases~~these studies, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose. More recent data from an ongoing AAV-FIX study demonstrates persistence of the vector in both the blood and the semen for at least 39 weeks (Miesbach, 2016, Haemophilia). ~~Viral shedding will also be extensively studied in the present clinical trial, every week up to 16 weeks, then every 4 weeks to one year, then every three months, until at least 3 consecutive negative results are obtained.~~ During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of ~~viral~~vector shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2, Table 9.1.3, and Table 9.1.4…. Vector shedding will also be extensively studied in the present clinical trial, every other week between Weeks 4-16, then every 4 weeks to Week 52 until at least 3 consecutive negative results are obtained. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor). Contraception use may need to be extended beyond 26 weeks in individual subjects based on observed vector shedding in semen.	4, 5, 6, 12

Section No./Title	Text Revisions	Rationale
	After 6 months, subjects may stop contraception use only if they have had 3 consecutive negative semen samples.	
10.2.1/Events of Special Interest (EOSI)	The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality: Elevation of liver enzymes (ALT) that triggers ~~a~~ an initiation or modification of corticosteroid treatment	12
10.4.1.3/Persistent or Recurrent Adverse Events	A persistent AE (duration of adverse event > 7 days) is one that extends continuously, without resolution, between subject evaluation time points. ~~Such~~Events that change in severity necessitate the recording of an ~~event~~ additional AE. AEs that do not have a change in severity should be recorded only once on the ~~AE~~ eCRF ~~unless its severity increases or decreases (in which case it should be recorded again on the AE eCRF).~~.	7, 12
10.4.1.4/Abnormal Laboratory Values	A clinical laboratory abnormality is considered clinically significant and should be documented as ~~AE if any~~an AE if not refuted by a repeat test to confirm the abnormality and **any** one or more of the following conditions is met: • ~~The laboratory abnormality persists upon repeat confirmatory testing.~~	12
10.9/BioMarin Pharmacovigilance Contact Inforamtion	Contact information for the medical monitor is as follows: Name: PI Address: 105 Digital Drive Novato, CA 94949 USA Phone: PI E-mail: PI	11
12.2/Screening Visit	Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, LFTs, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline. The following procedures will be performed during the Screening Period: • Complete Physical Exam ~~(including height and weight)~~ • Height and weight	1, 3, 8, 12

Section No./Title	Text Revisions	Rationale
	• Blood chemistry, haematology, ~~urine tests,~~ coagulation screen, and CRP (refer to Table 9.7.8.2.1) • Urine Tests (refer to Table 9.7.8.2.1) • Liver Function Tests (refer to Table 9.7.8.3.1) • Von Willebrand Factor Antigen (VWF:Ag)	
12.2.1/"Smart Rescreening" Visit	If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study: • Blood chemistry, haematology, ~~urine tests,~~ coagulation screen, and CRP (refer to Table 9.7.8.2.1) • Urine Tests (refer to Table 9.7.8.2.1) • Liver Function Tests (refer to Table 9.7.8.3.1)	1, 3, 12
12.3/Baseline Visit	The following procedures will be performed during the Baseline Period: • Blood chemistry, haematology, ~~urine tests,~~ coagulation screen, and CRP (refer to Table 9.7.8.2.1) • Urine Tests (refer to Table 9.7.8.2.1) • Liver Function Tests (refer to Table 9.7.8.3.1) • Direct Thrombin test • PCR of vector ~~genomes~~DNA in blood, saliva, urine, semen, and stools	1, 3, 10, 12
12.5.1/BMN 270 Infusion Follow-Up Visits/Once per Week	The following procedures will be performed at one visit per week from Weeks 1 through 16: • ~~Blood chemistry, haematology, urine tests, coagulation screen, and CRP~~ • Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment o ~~Each subject will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a subject's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.~~	1, 3

Section No./Title	Text Revisions	Rationale
	○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • Samples for FVIII Assays – central laboratory assessment ○ FVIII activity level (APTT) ○ FVIII activity level (chromogenic FXa assay) ○ FVIII coagulation activity exploratory assay ○ Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level ○ FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. Central assessment of FVIII activity level should be performed 1x/week from Week 1 through Week 16	
12.5.2/Twice per week (Weeks 1 through 16)	The following procedures will be performed twice per week from Weeks 1 through 16: • Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment ○ Local assessment of liver function tests should be performed on Day 8 during Week 1, and then 2x/week from Week 2 through Week 16 ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII activity level – local laboratory assessment ○ FVIII activity level (APTT) ○ FVIII activity level (chromogenic FXa assay) ▪ Local assessment of FVIII activity level should be performed on Day 8 during Week 1, and then 2x/week from Week 2 through Week 16	1, 3
12.5.3/Week 1 – Day 2 and Day 4	On Day 2 and Day 4 of Week 1, the following procedures will be performed: • PCR of vector ~~genomes~~DNA in blood, saliva, urine, semen, and stools (Day 2 and Day 4)	1, 3, 12

Section No./Title	Text Revisions	Rationale

	• Samples for FVIII Assays (Day 4 only) – central laboratory assessment
	  ○ FVIII activity level (APTT)
	  ○ FVIII activity level (chromogenic FXa assay)
	  ○ FVIII coagulation activity exploratory assay
	  ○ Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
	  ○ FVIII antigen (ELISA)
	Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
	• Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – central laboratory assessment
	• Liver Function Tests (refer to Table 9.7.8.3.1) (Day 4 only) – local laboratory assessment
	• FVIII activity level (Day 4 only) – local laboratory assessment
	  ○ FVIII activity level (APTT)
	  ○ FVIII activity level (chromogenic FXa assay)	

12.5.4/Week 1 – Day 8	On Day 8, the following procedures will be performed: • PCR of vector DNA in blood, saliva, urine, semen, and stools • Direct Thrombin test • Haemo-QoL-A QoL assessment	4, 10, 12
12.5.5/Every 2 Weeks	Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following ~~procedure~~ procedures will be performed: • Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) (not assessed at Week 14) • ~~Exploratory biomarker assessments~~ • PCR of vector DNA in blood, saliva, urine, semen, and stools (not collected at Week 2) ○ Collection to occur until at least 3 consecutive negative results are obtained	4, 9

Section No./Title	Text Revisions	Rationale
~~12.5.5/Weeks 1, 3, and 6~~	~~At Weeks 1, 3, and 6, the following procedure will be performed:~~ • ~~Test for Hepatitis B and Hepatitis C reactivation~~	1, 3, 12
12.5.6/Weeks 4, 8, 12, and 16	At Weeks 4, 8, 12, and 16, the following procedure will be performed: • PBMC collection • Urine Tests (refer to Table 9.7.8.2.1~~)~~ • VWF:Ag • FVIII antibody titer • Exploratory biomarker assessments	8, 12
12.5.7/Week 16	At Week 16, the following procedure will be performed: • Test for Hepatitis B and Hepatitis C reactivation Subjects will be tested for hepatitis B and hepatitis C reactivation at Week 16. Subjects who receive therapeutic corticosteroids prior to Week 16 do not need to complete the Week 16 reactivation assessment; instead, they will be tested for hepatitis B and hepatitis C reactivation at the time points listed in Table 9.1.5.	1, 3, 12
12.5.10/Weeks ~~1,~~ 2, 3, 4 and 16	At Weeks ~~1,~~ 2, 3, 4, and 16, the following procedure will be performed: • Haemo-QoL-A QoL assessment	12
12.5.11/Week 13	At Week 13, the following procedure will be performed: • Direct Thrombin test	10
~~12.6/Safety Follow-Up – Year 1~~	Section 12.6 and its subsections (describing assessments to be performed during the Week 17-52 Safety Follow-Up Period) have been deleted and replaced with the new Section 12.6 (for Weeks 17-36) and Section 12.7 (for Weeks 37-52).	12
12.6/Safety Follow-Up – Weeks 17-36	After the ~~16 weekly~~ Post-Infusion Follow-Up visits are complete, subjects will return to the study site ~~at~~for Safety Follow-Up visits from Weeks ~~20, 28,~~17 through Week 36~~, 44, and 52 (± 1 week~~ (± 48 hours), when the following procedures will be completed:	12

Section No./Title	Text Revisions	Rationale

12.6.1/Once per week (Weeks 17 through 36)	Once per week from Week 17 through Week 36, the following procedures will be performed:
	• Physical exam
	• Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
	• Vital Signs
	• ~~Blood chemistry, haematology, urine tests, coagulation screen, and CRP~~Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment
	  ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
	• FVIII Assays – central laboratory assessment
	  ○ FVIII activity level (APTT)
	  ○ FVIII activity level (chromogenic FXa assay)
	  ○ FVIII coagulation activity exploratory assay
	  ○ Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
	  ○ FVIII antigen (ELISA)
	Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
		3, 12

12.6.2/Twice per week (Weeks 17 through 20)	Twice per week from Week 17 through Week 20, the following procedures will be performed:
	• Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
	  ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
	• FVIII activity level – local laboratory assessment
	  ○ FVIII activity level (APTT)
	  ○ FVIII activity level (chromogenic FXa assay)
		3

Section No./Title	Text Revisions	Rationale

12.6.3/Once per week (Weeks 21 through 36)	Once per week from Week 21 through Week 36, the following procedures will be performed:
	• Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment
	  ○ LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data.
	• FVIII activity level – local laboratory assessment
	  ○ FVIII activity level (APTT)
	  ○ FVIII activity level (chromogenic FXa assay)
		3

12.6.4/Every 4 Weeks	Every 4 weeks (Weeks 20, 24, 28, 32, and 36), the following procedures will be performed: • Exploratory biomarker assessments (Weeks 20 and 24 only) • Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) • Urine Tests (refer to Table 9.7.8.2.1) • VWF:Ag • AAV5 antibody titer • PBMC collection • PCR of vector DNA in blood, saliva, urine, semen, and stools ○ Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.	5, 8, 9
12.6.5/Every 8 Weeks	Every 8 weeks (Weeks 20, 28, and 36), the following procedure will be performed: • FVIII antibody titer	12
12.6.6/Week 26	At Week 26, the following procedure will be performed: • Direct Thrombin test	10

Section No./Title	Text Revisions	Rationale
	• Weight	
12.6.7/Weeks 28	At Week 28, the following procedure will be performed: • Haemo-QoL-A QoL assessment	12
12.7/Safety Follow-up – Weeks 37-52	Subjects will return every 2 weeks (Week 38, 40, 42, 44, 46, 48, 50, and 52) from Week 37-52 (± 1 week), when the following procedures will be completed:	12
12.7.1/Once per visit	At Weeks 38, 40, 42, 44, 46, 48, 50, and 52, the following procedures will be performed: • Physical exam • Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) • Vital Signs • Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment o LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII activity level – local laboratory assessment o FVIII activity level (APTT) o FVIII activity level (chromogenic FXa assay) • Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment o LFTs may be monitored more or less frequently based (and in particular when ALT values are >1.5x ULN) on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII Assays – central laboratory assessment o FVIII activity level (APTT)	3, 12

Section No./Title	Text Revisions	Rationale
	○ FVIII activity level (chromogenic FXa assay) ○ FVIII coagulation activity exploratory assay ○ Bethesda assay (with Nijmegen modification) for FVIII inhibitor level ○ FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved	
12.7.2/Every 4 Weeks	Every 4 weeks (Weeks 40, 44, 48, and 52), the following procedures will be performed: • Blood chemistry, haematology, coagulation screen, and CRP (refer to Table 9.7.8.2.1) • Urine Tests (refer to Table 9.7.8.2.1) • AAV5 antibody titer • VWF:Ag • PCR of vector ~~genomes~~DNA in blood, saliva, urine, semen, and stools Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. Subjects who have not had 3 consecutive negative samples by Week 52 should continue to have PCR testing every 4 weeks until 3 consecutive negative samples are documented (or upon consultation between the Investigator and Medical Monitor).	5, 8, 9, 12
12.7.3/Every 8 Weeks	Every 8 weeks (Weeks 44 and 52), the following procedure will be performed: • PBMC collection • FVIII antibody titer	12
12.7.4/Week 38 and 52	At ~~Weeks 28 and~~Week 38 and Week 52, the following procedure will be performed: • Direct Thrombin test	10
12.7.5/Week 52	At Week 52, the following procedure will be performed: • Haemo-QoL-A QoL assessment	12

Section No./Title	Text Revisions	Rationale
	• Weight	
12.8.1/Safety Follow-Up (Years 2-5): Every Visit	Every 3 months (± 2 weeks), the following procedures will be performed: • Vital Signs • Blood chemistry, haematology, ~~urine tests,~~ coagulation screen, and CRP (refer to Table 9.7.8.2.1) • Urine Tests (refer to Table 9.7.8.2.1) • Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment o LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII activity level – local laboratory assessment o FVIII activity level (APTT) o FVIII activity level (chromogenic FXa assay) • Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment o LFTs may be monitored more or less frequently (and in particular when ALT values are >1.5x ULN) based on discussion between the Medical Monitor and the Investigator and review of subject data. • FVIII Assays – central laboratory assessment o FVIII activity level (APTT) o FVIII activity level (FXa chromogenic assay) o FVIII coagulation activity exploratory assay o Bethesda assay (with Nijmegen modification) for FVIII inhibitor level o FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. • VWF:Ag	1, 3, 8, 10, 12

Section No./Title	Text Revisions	Rationale
	• Direct Thrombin test • ~~PCR of vector genomes in blood, saliva, urine, semen, and stools~~ o ~~Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.~~ • ~~Vital Signs~~	
12.8.2/Every Other Visit (Every 6 Months)	• Weight	
12.9/Early Termination Visit	At the Early Termination visit, as many of the following assessments as possible should be done: • Vital Signs • Blood chemistry, haematology, ~~urine tests,~~ coagulation screen, and CRP (refer to Table 9.7.8.2.1) • Urine Tests (refer to Table 9.7.8.2.1) • Liver Function Tests (refer to Table 9.7.8.3.1) – local laboratory assessment • FVIII activity level – local laboratory assessment o FVIII activity level (APTT) o FVIII activity level (chromogenic FXa assay) • Liver Function Tests (refer to Table 9.7.8.3.1) – central laboratory assessment • FVIII Assays – central laboratory assessment o FVIII activity level (APTT) o FVIII activity level (chromogenic FXa assay) o FVIII coagulation activity exploratory assay o Bethesda assay (with Nijmegen modification) for FVIII inhibitor level o FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII	1, 3, 5, 6, 8, 10, 12

Section No./Title	Text Revisions	Rationale
	activity measurements when a 72-hour interval without FVIII use is achieved. • VWF:Ag • Direct Thrombin test • PCR of vector ~~genomes~~DNA in blood, saliva, urine, semen, and stools o Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. • ~~Vital Signs~~	
14.2/Primary and Secondary Efficacy Analysis	Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay as measured by the central laboratory at 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.	1, 3, 12
14.6/Determination of Sample Size	The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to ~~12~~15 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.	2

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI , MD, PhD PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Subject Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015
Date of Amendment 3:	06 November 2015

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 3

Date: 06 November 2015

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 3 to the BMN 270-201 protocol is provided below.

1. If a subject develops hepatic transaminases elevated 1.5x above his baseline during the study, changes to subject management, including the use of prophylactic glucocorticoids and a change to the frequency of liver function testing, will be adopted.

Rationale: Previous clinical trials using AAV2-FIX and AAV8-FIX have shown some occurrences of elevation of liver enzymes 5-12 weeks after dosing. Such elevation was correlated with a loss of transgene expression in these clinical trials. As the present trial is a First-in-Human (FIH) for the combination of this vector serotype and Factor VIII, the occurrence of such a liver response is unknown as well as the possible consequences on gene expression. Occurrences of liver enzyme elevation in previous clinical trial were readily managed by a tapered regimen of low dose steroids. By including prophylactic corticosteroids for all subsequent subjects as well as shifting the surveillance monitoring period to coincide with the initial liver response, reductions in transgene expression may be avoided.

2. Added as an exclusion criterion any subject with major surgery planned during the 16-week period following viral infusion.

Rationale: A major surgery, such as hip replacement, planned during the initial 16-week follow up period would jeopardize the subject's safety, as it is unknown what his expression level of factor VIII would be at this time.

3. Added a "smart rescreening" option for subjects who are successfully screened but do not undergo Baseline assessments and Infusion within the 28 + 14 day window required by the protocol.

Rationale: Reducing the number of screening assessments for a smart rescreen poses minimal risk to the subject and keeps the subject below the statutory 12 week blood volume threshold. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not

complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

4. Clarified that subjects who have cleared a Hepatitis B infection or are seronegative will not be required to receive the hepatitis vaccination as a requirement for enrolling in the study.

Rationale: Subjects who have cleared the infection or are seronegative have a very low chance of becoming seropositive during the trial. Most Haemophilia A patients are already vaccinated, and the risk is too low to mandate the vaccination.

5. Minor edits for clarity and consistency have been incorporated.

Specific changes included in this amendment, including the Synopsis, since Amendment 2 (approved 26 May 2015) are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

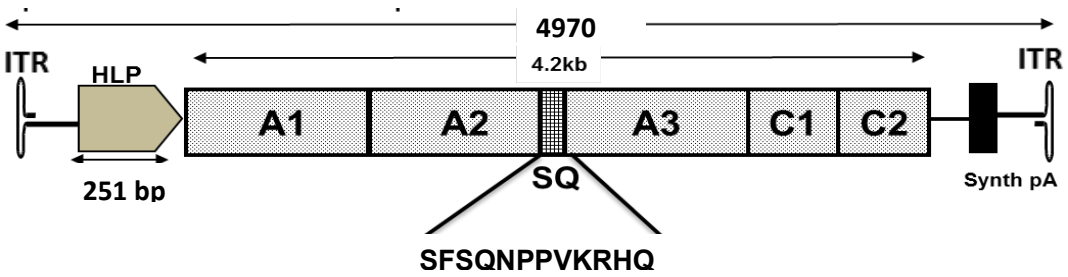
Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	Referring to Part of the Dossier: Volume: Page: Reference:	

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 1).

Figure 1. BMN 270 transgene construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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 expression of 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

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STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to three cohorts according to dose level:

1. 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
2. 2E13 vg per kilogram, iv
3. 6E13 vg per kilogram, iv

Cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the aPTT and chromogenic assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses.

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study, the Data Review Board (DRB).

If the FVIII activity reaches ≥ 5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Subject 1 will be dosed by intravenous infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week

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3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each Cohort, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort. Although the optimum design of this study is three cohorts of 4 subjects, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC).

NUMBER OF SUBJECTS PLANNED:

Up to 12 subjects may enroll into the study; the actual number of subjects will depend on the FVIII activity levels seen in each Cohort.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months

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6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any vector or gene transfer agent

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15. Major surgery planned in the 16-week period following the viral infusion
16. Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each subject will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous infusion.

CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver function tests (LFTs, including ALT, AST, GGT, LDH, bilirubin, alkaline phosphatase)
- Immune response to FVIII transgene and AAV capsid proteins

No major toxicity is expected based on preclinical studies in mice and monkeys. An asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan. Each subject will have comprehensive surveillance monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a subject's ALT is found to be greater than 1.5x his baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension; the frequency and duration of LFT testing

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may be changed if dictated by ongoing safety evaluations.

There will be a detailed assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Subjects will be asked to keep a subject diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 12 subjects may be dosed in the study. The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay at 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as viral shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
DMC	Data Monitoring Committee
DRB	Data Review Board
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
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
HA	Haemophilia A
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee
IND	Investigational New Drug (application)
INR	international normalized ratio

IP	investigational product
IRB	institutional review board
IV	intravenous
LFT	liver function test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
vg	vector genomes

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing

eligible subjects for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each subject will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF , and if applicable, a copy of the approved subject information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the record file of the subject.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to subjects. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Liver function tests (LFTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LFTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to 18-19 hours (Kaufman, 2013, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration (Hay, 2012, Blood).

Gene therapy offers the potential of a cure through continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2013, Haemophilia.); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This

nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in

the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Nathwani, 2006, Blood). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents 10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical toxicology study in mice.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Engl.J.Med.), (Nathwani, 2014, N.Engl.J.Med.). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is being tested in other clinical trials and was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola, 2014, J.Hepatol.). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Mannucci, 2001, N.Engl.J.Med.). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. . The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

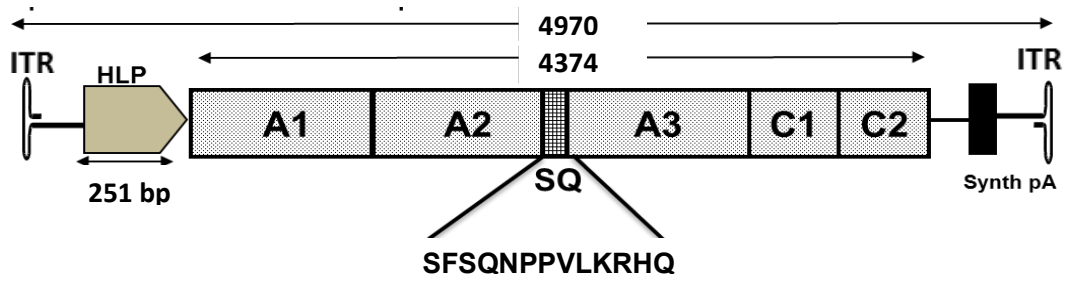
Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011, Haemophilia.). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia.), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Mannucci, 2001, N.Engl.J.Med.). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol.Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, 2014, N.Engl.J.Med.). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.), (Bainbridge, 2008, N.Engl.J.Med.), (Maguire, 2009, Lancet); (Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: BMN 270 Transgene Construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of FVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan (Nathwani, 2011, Mol.Ther.). Each subject will have a comprehensive surveillance plan that monitors LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a subject's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg. A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to subjects with the best chance of benefiting the subject therapeutically.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled into one of up to three cohorts according to dose level:

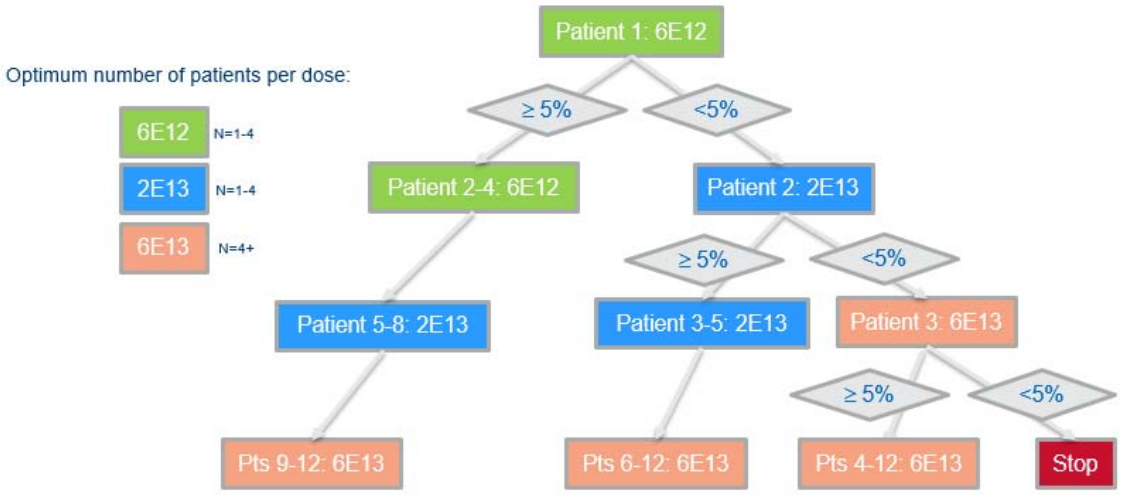
1. 6E12 vg/kg of body weight, given as a single intravenous dose (iv)
2. 2E13 vg/kg, iv
3. 6E13 vg/kg, iv

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in mice.

Cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity reaches≥5 IU/dL at the Week 3 visit, then the remaining subjects in the cohort will be enrolled without the need to wait for 3 weeks between subjects.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme



Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each Cohort, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort. Although the optimum design of this study is three cohorts of 4 subjects, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals. Because subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dosing. Any safety signal may trigger a review of the data and possible additional immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear cells (PBMC). Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to

complete an extensive safety analysis. Notification of the study halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the Data Review Board (DRB) and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 for Safety Follow-up.

Table 9.1.4, dealing with steroid prophylaxis in the event of elevated LFTs, is discussed in Section 9.4.8.2.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion			BMN 270 Infusion Visit (Day 1)[k]
	Screening[i] (Day -28 to Day -1)	**Smart Rescreening[i] (Day -28 to Day -1)**	**Baseline (Day -7 to Day -1)[h]**	
Informed consent	X			
Medical History	X			
Physical Exam[a]	X		X	X
Vital Signs	X	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X
Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)	X	X	X	
Distribution of subject diaries and training in their use			X	
Electrocardiogram	X			
Chest X-ray	X			
Liver Ultrasound	X			
hFVIII Assays[b]	X	X[j]		
AAV5 Assays[c]	X	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X			
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[e]	X	X	X	
PBMC collection for CTL baseline			X	
PCR of vector genomes in blood, saliva, urine, semen, and stools			X	
Biomarker testing[f]	X			
Exploratory biomarker assessments[g]			X	
Haemo-QoL-A Quality of Life (QoL) assessment			X	
BMN 270 Infusion				X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height and weight will be recorded at Screening only.

[b] Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). The assessment on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[f] Includes HLA genotyping, FVIII genotyping, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

[i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

[j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening.

[k] Assessments on the day of infusion should be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up

Assessment	Follow-Up After BMN 270 Administration - Weeks*																	
	Week 1			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	D2	D4	D8															
Physical exam[a]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII assays[c]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FVIII antibody titer			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Exploratory biomarker assessments[e]				X		X		X		X		X		X		X		X
Haemo-QoL-A QoL assessment			X	X	X	X												X
AAV5 antibody titer										X								X
Testing for reactivation of hepatitis B and hepatitis C			X		X			X										
PBMC collection						X				X				X				X

* Visit windows are ±48 hours (and include the Day 4 visit)

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included. Each subject will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a subject's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

[c] Includes hFVIII activity level (APTT and FXa chromogenic assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

[d] Collection to occur on Day 2 and 4 following BMN 270 infusion, and then weekly until at least 3 consecutive negative results are obtained.

[e] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

Table 9.1.3: Schedule of Events – Safety Follow-Up

Assessment	Year 1 - Weeks*					Years 2-5*	ETV (early termination visit)
	20	28	36	44	52	Q3M	
Physical exam[a]	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]	X	X	X	X	X	X	X
FVIII assays[c]	X	X	X	X	X	X	X
AAV5 antibody titer	X	X	X	X	X	X	X
FVIII antibody titer	X	X	X	X	X	X	X
PBMC Collection (for determination of FVIII and Capsid specific CTL activity)	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X
Haemo-QoL-A QoL assessment		X			X	X[e]	X

* Visit windows are ±1 week for visits in Year 1, and ± 2 weeks for visits in Years 2-5.

[a] Complete physical examination should be performed at Weeks 52 and every 52 weeks thereafter; brief physical exam may be performed at other study visits. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved.

[d] Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[e] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).

Table 9.1.4: Schedule of Events – Steroid Prophylaxis

	Week 3	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11**	**Week 12**	**Week 13**	**Week 14**	**Week 15**	**Week 16**	**Week 17**	**Week 23-25**
Prophylactic steroids (dose in mg/day) [a]	40 mg	40 mg	40 mg	40 mg	35 mg	35 mg	30 mg	30 mg	25 mg	25 mg	20 mg	20 mg	15 mg	10 mg	5 mg	
Hepatitis B testing[b]																X
Liver function testing[c]	Weekly, then 3x/week during the 6-week period when a liver response is expected based on the liver response in the original subject															

[a] Steroid prophylaxis will be started in all subjects not yet dosed following the first subject who develops elevated liver function tests (defined as 1.5x baseline ALT) during the study. Subjects already dosed who have not yet reached Week 17 in the study will be evaluated for the use of prophylactic steroids after discussion between the Investigator and the Medical Monitor. Deviations from this prophylactic steroid dosing regimen must be approved by the Medical Monitor.

[b] The subject's hepatitis B status should be rechecked 6-8 weeks after the end of corticosteroid treatment, using the same tests performed for hepatitis B status at Screening.

[c] Reports of abnormal LFTs (defined as 1.5x the subject's baseline ALT) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available.

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Three doses of BMN 270 will be evaluated and the dose escalation decision tree is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

There will be no control group. Parameters for each subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 12 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, and results from a modified Nijmegen Bethesda assay of less than 0.6 Bethesda Units (BU) 2 consecutive occasions at least one week apart within the past 12 months
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition or AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. HIV positive
4. Significant liver dysfunction as defined by abnormal elevation of:
   - ALT (alanine transaminase) to 3 times the upper limit of normal;
   - Bilirubin above 3 times the upper limit of normal;
   - Alkaline phosphatase above 3 times the upper limit of normal; or
   - INR (international normalized ratio) ≥ 1.4.
5. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
6. Evidence of any bleeding disorder not related to Haemophilia A
7. Platelet count of $< 100 \times 10^9$/L
8. Creatinine ≥ 1.5 mg/dL
9. Liver cirrhosis of any etiology as assessed by liver ultrasound
10. Hepatitis B if surface antigen is positive
11. Hepatitis C if RNA is positive
12. Treatment with any IP within 30 days prior to the end of the screening period
13. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
14. Prior treatment with any gene transfer agent
15. Major surgery planned in the 16-week period following the viral infusion
16. Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject requires medication or medical procedure prohibited by the protocol
- Subject does not adhere to study requirements specified in the protocol
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone, a certified letter should be sent to the subject requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each subject. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur in a subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- A 5-fold increase from baseline in ALT after BMN 270 administration
- The occurrence of Grade 3 or higher adverse events assessed as related to study drug, including liver failure and clinical hepatitis
- Grade 2 adverse event assessed as related to study drug that persists for at least 7 days
- The detection of neutralizing antibodies to hFVIII following BMN 270 infusion
- The persistent detection of the AAV vector genome in the semen of a participant more than 26 weeks after BMN 270 infusion, as discussed in Section 9.7.8.5
- The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion

9.3.4 Subject Identification and Replacement of Subjects

Each subject will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who withdraw from the study will not be replaced.

9.3.5 Duration of Subject Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial. Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical exam performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (e.g. the median cubital vein) and flushed with saline. FVIII replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours, although the IP has been shown to be stable at room temperature for 6 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Subjects will remain in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours, participants will be discharged from the clinic unless toxicity has been observed in which case the stay in the clinic may be extended or the subject may transfer to a separate facility based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Subjects to Treatment Groups

Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study subject. Upon their enrollment into the study, subjects will be assigned a unique subject number and dose level by the Sponsor.

Cohorts are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Cohorts may receive the next higher dose if subjects in the previous cohort does not meet the activity criteria, or the same dose if subjects in the previous cohort meets the activity criteria.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in mice. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Subject

A minimum of three weeks are required between subjects, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next subject will be made as described below.

9.4.6.2 Selection of Dose for Each Subject

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and the DRB.

Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 vg/kg of body weight. If the FVIII activity level does not reach 5 IU/dL or greater (expressed as % in Figure 9.1.1) at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg/kg).

If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject.

If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.

For the first subject treated in each Cohort, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four subjects will be enrolled in the Cohort. Although the optimum design of this study is three cohorts of 4 subjects, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels of FVIII and safety signals.

Refer to Figure 9.1.1 for a visual representation of the study design.

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Systemic immunosuppressive agents

9.4.8.1 Concomitant Haemophilia Treatments

Subjects on "on demand" therapy for FVIII will continue their treatment at will. Subjects on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the subject, who will carefully record his treatment and bleeding episodes in his diary.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period, each subject will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a subject's ALT is

found to be greater than 1.5x his baseline during these periods, then LFTs will be monitored three times per week until the ALT returns to baseline.

Reports of abnormal LFTs (defined as 1.5x the subject's baseline ALT level) should be made to the BioMarin medical monitor within 30 minutes of the lab value being available, and treatment with prednisolone will be initiated immediately at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first 2 weeks, then 40 mg the next 2 weeks, then 30 mg the next two weeks, then 20 mg the next two weeks, then stop, for a total treatment of 8 weeks). Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.

If a glucocorticoid treatment is started for a subject at any point of the study, the following steps will be taken:

- A prophylactic glucocorticoid treatment will be started for subsequent subjects scheduled to be dosed. Subjects will receive prednisolone 40 mg per day starting at week 3, for a duration of 4 weeks, then tapered by 5 mg every two weeks for 8 weeks (until 20 mg/day is reached), then tapered 5 mg per week for 3 weeks, for a total duration of 15 weeks (refer to 4).
    - Subjects already dosed who have not yet reached Week 17 in the study will be evaluated for the use of prophylactic steroids after discussion between the Investigator and the Medical Monitor.
- Once prophylactic glucocorticoid treatments have been initiated, then all subjects (who have not already passed that period in the study) will have 3x/week LFT assessments during the 6-week period when a liver response is expected based on the liver response in the original subject, if this period is different from the 3x/weekly LFT assessments already planned for Weeks 7-12.
    - Subjects who have already passed that 6-week period in the study will continue to have the regularly scheduled LFT assessments as planned in the protocol.
- Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. Hepatitis B status will be rechecked at the end of the corticosteroid treatment. All adverse events should be reported as outlined in Section 10 of the protocol.

9.4.9 Treatment Compliance

Study drug will be administered to subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return or destroy all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to stable expression of FVIII at 5 IU/dL or above, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes, (Den Ujil, 2011, Haemophilia).

The following assays will be used to measure the primary efficacy variable:

- FVIII activity (chromogenic FXa assay)
- FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time)

If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

The FVIII activity level in both assays and the number of subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Subjects will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the subject's diary or other subject records.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage APTT and/or chromogenic FXa assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2 and Table 9.1.3.

9.7.6 Exploratory Biomarker Assessments

Blood samples will be collected from subjects at the time points indicated in Table 9.1.1 and Table 9.1.2 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed. No additional genetic or genomic testing will be conducted on any sample (other than testing specified in the protocol).

9.7.7 Haemo-QoL-A Quality of Life Assessment

The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the subject signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	Haematology	Urine Tests	Other
Albumin	Haemoglobin	Appearance	CRP
Alkaline phosphatase	Haematocrit	Color	
ALT (SGPT)	WBC count	pH	**Coagulation Screen including:**
AST (SGOT)	RBC count	Specific gravity	APTT
Direct bilirubin	Platelet count	Ketones	PT/INR
Total bilirubin	Differential cell count	Protein	TT
BUN		Glucose	
Calcium		Bilirubin	
Chloride		Nitrite	
Total cholesterol		Urobilinogen	
CO_2		Haemoglobin	
Creatinine			
Glucose			
GGT			
LDH			
Phosphorus			
Potassium			
Total protein			
Sodium			
Uric acid			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

9.7.8.3 Liver function and Hepatitis Testing at Screening

Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. Subjects with documented results showing an absence of active Hepatitis B or Hepatitis C infection (as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) 30 days prior to providing signed informed consent do not need to be screened again.

Evidence of ongoing Hepatitis B or Hepatitis C infection is exclusionary. Subjects who have cleared a Hepatitis B infection or are seronegative do not need to receive the Hepatitis B vaccination.

A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- More than 3x the normal Alkaline phosphatase level.
- INR $\geq$ 1.4.
- Thrombocytopoenia under 100 x 10^9/L
- Liver ultrasound results indicative of a liver cirrhosis

9.7.8.4 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).

A complete physical examination is necessary during Screening/Baseline; thereafter, brief physical examinations may be performed at the discretion of the investigator based on the subject's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems.

A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, and gastrointestinal assessments.

Weight and height will be recorded at Screening only.

9.7.8.5 Viral Shedding

Viral shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Engl.J.Med.); (Manno, 2006, Nat.Med.); (Schenk-Braat, 2007, J.Gene Med.); (Croteau, 2004, Ann.Occup.Hyg.). In the literature referenced above, vector was no longer detectable after 40 days in blood, saliva, urine or stool. However, vector persisted in semen for 16 weeks in the seminal fluid but not in motile sperm (Manno, 2006, Nat.Med.). In all cases, the amount of vector present in these bodily fluids involved an extremely small fraction of the infused dose.

Viral shedding will also be extensively studied in the present clinical trial, every week up to 16 weeks, then every 4 weeks to one year, then every three months, until at least 3 consecutive negative results are obtained. During the Post-Infusion Follow-Up period, subjects will undergo testing of various bodily samples to look for evidence of viral shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2 and Table 9.1.3. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the subject is a haemophiliac) should not be recorded as AEs but will instead be captured in subject diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of major trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening

  Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the subject or require intervention to prevent one of the above consequences (e.g. anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of liver enzymes (ALT) that triggers a corticosteroid treatment

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs, SAEs, and EOSI at each subject contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs

and EOSI, should be recorded in the subject's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.2. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred • OR • The administration of the IP and the occurrence of the AE are not reasonably related in time • OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time **AND** • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time **AND** • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology

- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between subject evaluation time points. Such an event should be recorded only once on the AE eCRF unless its severity increases or decreases (in which case it should be recorded again on the AE eCRF).

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as AE if **any** of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The laboratory abnormality persists upon repeat confirmatory testing.
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type

explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a subject taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in partner should be reported within 24 hours of the site becoming aware of the pregnancy by entering the information on the Pregnancy eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the subject's partner through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BPV within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Subjects after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the subject's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each subject to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax: PI
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI, MD, PhD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic FXa assay and the APTT assays are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, Hepatitis B, Hepatitis C, and HIV.
- Complete Physical Exam (including height and weight)
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic FXa (plasma)
    - Baseline hFVIII activity level – one-stage APTT assay
    - hFVIII coagulation activity exploratory assay
    - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
    - hFVIII total antibody assay
    - hFVIII antigen (ELISA)

- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Screen for Hepatitis B, Hepatitis C, and HIV if required (subjects with documented negative results 30 days prior to informed consent being obtained do not need to be retested)
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Blood samples for Biomarker testing (including HLA genotyping, FVIII genotyping status, TNFα and IL10a single nucleotide polymorphisms)

12.2.1 "Smart Rescreening" Visit

Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once.

If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study:

- Vital signs
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
- Blood sample for AAV5 Assays
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
- Distribution of subject diaries and training in diary completion
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- PBMC collection for CTL baseline
- PCR of vector genomes in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- Haemo-QoL-A QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each subject. Subjects will remain in the clinic for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assay
- BMN 270 Infusion
- Vital Signs
    - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the subject's stay in the clinic.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, subjects will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Every Visit

Every week (Weeks 1 through 16), the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
    - Each subject will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a subject's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.
- Samples for FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- FVIII antibody titer
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Collection to occur weekly until at least 3 consecutive negative results are obtained

12.5.2 Week 1 – Day 2 and Day 4

On Day 2 and Day 4 of Week 1, the following procedures will be performed:

- PCR of vector genomes in blood, saliva, urine, semen, and stools (Day 2 and Day 4)
- Samples for FVIII Assays (Day 4 only)
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.

12.5.3 Every 2 Weeks

Every 2 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedure will be performed:

- Exploratory biomarker assessments

12.5.4 Weeks 4, 8, 12, and 16

At Weeks 4, 8, 12, and 16, the following procedure will be performed:

- PBMC collection

12.5.5 Weeks 1, 3, and 6

At Weeks 1, 3, and 6, the following procedure will be performed:

- Test for Hepatitis B and Hepatitis C reactivation

12.5.6 Weeks 8 and 16

At Weeks 8 and 16, the following procedure will be performed:

- AAV5 antibody titer

12.5.7 Weeks 1, 2, 3, 4, and 16

At Weeks 1, 2, 3, 4, and 16, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.6 Safety Follow-Up – Year 1

After the 16 weekly infusion follow-up visits are complete, subjects will return to the study site at Weeks 20, 28, 36, 44, and 52 (± 1 week), when the following procedures will be completed:

12.6.1 Every Visit

At Weeks 20, 28, 36, 44, and 52, the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.2 Weeks 28 and 52

At Weeks 28 and 52, the following procedure will be performed:

- Haemo-QoL-A QoL assessment

12.7 Safety Follow-Up – Years 2-5

During Years 2-5 of Safety Follow-up, subjects will be assessed every 3 months (± 2 weeks). At these times, the following procedures will be completed:

12.7.1 Every Visit

Every 3 months (± 2 weeks), the following procedures will be performed:

- Physical exam
    - Complete Physical Exam will be performed every 52 weeks; Brief Physical Exams may be performed at other visits.
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (FXa chromogenic assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs

12.7.2 Every Other Visit (Every 6 Months)

Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedure will be performed:

- Haemo-QoL-A QOL assessment

12.8 Early Termination Visit

The Early Termination visit will occur on the date the subject withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a subject leaves the study prior to the Week 260 visit, the subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use)
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII antigen (ELISA)

  Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.
- AAV5 antibody titer
- FVIII antibody titer
- PBMC collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs

- Haemo-QoL-A QOL assessment

12.9 End of Study

The study will end after the last subject completes the last Safety Follow-Up visit (Week 260). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study subjects may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all subjects having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The subjects will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay at 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons

14.4 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma

profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.5 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of subjects who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.6 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 12 subjects may be dosed in the study; the actual number of subjects will depend on the criteria for dose escalation.

14.7 Analysis Populations

The Safety analysis population is defined as all enrolled subjects who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled subjects who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA REVIEW BOARD

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available subject safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of subject safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon

completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB/IEC/REB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201, Amendment 3

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

PI

Printed name: PI , MD, PhD, PI , Clinical Sciences_____

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See pages 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Text Revisions	Rationale
2/Synopsis/Study Rationale	Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for ~~rhFVIII (under 24~~FVIII (12 to 18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment).	1, 5
2/Synopsis/Study Design and Plan	~~Patients~~Cohorts will be enrolled sequentially ~~every~~, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single ~~patient~~subject has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both the aPTT and chromogenic assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the ~~patient~~subject numbers exposed to subtherapeutic doses. Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study~~.~~, the Data Review Board (DRB). If the FVIII activity ~~is~~reaches ≥ 5 IU/dL at the Week 3 visit, then the ~~other patients of~~remaining subjects in the ~~dose group~~cohort will be enrolled without ~~waiting~~the need to wait for 3 weeks between ~~patients~~subjects. ~~Patient~~ Subject 1 will be dosed by intravenous ~~perfusion~~ infusion with 6E12 vector genomes [vg] per kilogram of body weight. If the FVIII activity level does not reach ≥ 5 IU/dL at ~~21 days~~ the Week 3 visit in both assays, then ~~a higher~~ no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg ~~per kilogram) will be used for the next patient.~~/kg). If the ~~activity level does not reach ≥ 5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.~~ FVIII activity level in the first subject treated in Cohort 2 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then no further subjects will be dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the Data Review Board (DRB) will recommend whether to continue to add subjects to the cohort and/or whether to continue the study.	5

Section No./Title	Text Revisions	Rationale
	For the first subject treated in each ~~dose~~ Cohort, if the activity level reaches ≥5 IU/dL and no safety issue is found, then up to four ~~patients will receive~~ subjects will be enrolled in the Cohort. Although the optimum design of this ~~dose. If at any time~~ study is three cohorts of 4 subjects, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels ~~reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.~~ Of FVIII and safety signals. Because ~~patients~~subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these ~~patients~~subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after ~~dose~~ dosing. Any safety signal ~~will~~ may trigger a review of the data and possible additional ~~analysis including~~ immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear ~~collection.~~ Cells (PBMC).	
2/Synopsis/Number of Subjects Planned	Up to 12 ~~patients~~subjects may enroll into the study; the actual number of ~~patients~~subjects will depend on the ~~criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level~~ VIII activity levels seen in each Cohort.	5
2/Synopsis/Diagnosis and All Criteria for Inclusion and Exclusion	Individuals eligible to participate in this study must meet all of the following criteria: • Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII ~~baseline~~ level is 1 IU/dL or less • Treated/exposed to FVIII concentrates or ~~cryoprecipitates~~ cryoprecipitate for a minimum of 150 exposure days (EDs) • No history of inhibitor, ~~or no inhibitor on 2 consecutive occasions within the past 12 months using~~ and results from a modified Nijmegen Bethesda assay ~~higher~~ of less than 0.6 Bethesda ~~Unit~~ Units (BU) on 2 consecutive occasions at least one week apart within the past 12 months Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: • Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition ~~and~~ora AAV5 total antibodies • ~~Patients that are~~ HIV positive ~~are excluded.~~ • Significant liver dysfunction as defined by abnormal elevation of:	1, 2, 5

Section No./Title	Text Revisions	Rationale
	○ ALT (alanine transaminase) to 3 times the upper limit of normal~~, bilirubin, ~~; ○ Bilirubin above 3 times the upper limit of normal; ○ Alkaline phosphatase~~,~~ above 3 times the upper limit of normal; or ○ ~~an~~ INR (international normalized ratio) ~~of~~≥ 1.4 • Treatment with any IP within 30 days prior to the end of the screening ~~visit~~ period • Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study~~.~~ including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion. • ~~Receipt of~~ Prior treatment with any vector or gene transfer agent • Major surgery planned in the 16-week period following the viral infusion • Use of systemic immunosuppressive agents or live vaccines within 30 days before the viral infusion	
2/Synopsis/Duration of Treatment	BMN 270 is given as a single dose by intravenous ~~perfusion~~ infusion.	5
2/Synopsis/Criteria for Evaluation	Safety: The following safety outcome measurements will be assessed: • Incidence of adverse events (AEs), including serious AEs (SAEs) • Change in clinical laboratory tests (serum chemistry and haematology) • Change in vital signs • Change in physical examination • Vector shedding • Liver function tests (~~LFT~~LFTs, including ALT, AST, GGT, LDH, bilirubin, alkaline phosphatase) • Immune response to FVIII transgene and AAV capsid proteins No major toxicity is expected based on preclinical studies in mice and monkeys. An asymptomatic transaminitis was observed at	1, 5

Section No./Title	Text Revisions	Rationale
	week ~~8 10~~7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan. Each ~~patient~~ subject will have comprehensive surveillance ~~plan~~ monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a ~~patient's~~ subject's ALT is found to be greater than 1.5x his baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension; the frequency and duration of LFT testing may be changed if dictated by ongoing safety evaluations. There will be a detailed ~~monitoring plan for~~ assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.	
2/Synopsis/Statistical Methods	Efficacy is a secondary endpoint, defined as biologically active FVIII at ≥ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay ~~between 1 to~~ at 16 weeks following study treatment.	5
4/List of Abbreviations	~~eCRF~~ CSR - ~~electronic case~~ clinical study report ~~form~~ eCRF - electronic case report form PBMC - peripheral blood mononuclear cells	5
6/Investigators and Study Administrative Structure	Liver function tests (LFTs) will be performed at the local laboratories associated with the study sites. Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed (refer to Section 9.4.8.2~~Laboratory evaluations will be performed at the local laboratories associated with the study sites.~~). In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action. Safety labs evaluations (including LFTs) will be performed at the central lab, while bioanalytical samples will be performed at the appropriate specialty lab. Refer to the Laboratory Manual for more details.	1
7/Introduction	Chemical modification or bioengineering of FVIII may improve half-life to ~~around 20~~18-19 hours ~~(Pipe, 2010, Blood),~~Kaufman, 2013, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration ~~or problems of FVIII inhibitor formation, which occurs in 30% of patients on standard FVIII replacement therapy (Nathwani, 1992, Baillieres Clin.Haematol.).~~(Hay, 2012, Blood).	5
7.1/Nonclinical Studies	BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in FVIII KO x Rag2 mice given matched IU levels of Refacto®. Additionally, the BMN 270 transgene product has a final sequence closely matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no	5

Section No./Title	Text Revisions	Rationale
	unique FVIII-specific target organs of toxicity. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (~~Paneda, 2009, Hum.Gene Ther.).~~Nathwani 2006, Blood). This dose represents ~~> 30~~10-fold safety factor from the no observed adverse effect level (NOAEL) in the GLP enabling nonclinical ~~studies~~ toxicology study in mice.	
7.2/Previous Clinical Studies	AAV serotype 5 is ~~also used~~ being tested in other clinical trials and ~~has shown good safety~~ was reportedly well tolerated without treatment-related serious adverse events in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola. 2014, J Hepatology). In addition, AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.	5
7.3/Study Rationale	Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (~~Nathwani, 1992, Baillieres Clin. Haematol~~ Mannucci, 2001, NEJM). Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant human FVIII protein (rhFVIII) concentrates both as prophylaxis 2-~~3~~4 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for ~~rhFVIII (under 24~~FVIII (12-18 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis and up to 30 to 50 episodes/year for on demand treatment) (Nagel, 2011, Haemophilia). Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (~~Pipe, 2010, Blood).~~Mannucci, 2001, NEJM). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector (Nathwani, ~~2011~~2014, NEJM). BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active ~~hFVIII~~ FVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these	1, 5

Section No./Title	Text Revisions	Rationale
	levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of ~~hFVIII~~ FVIII in patients with severe HA.	
7.4/Summary of Overall Risks and Benefits	Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week ~~8-10~~7-12 after administration in humans with an AAV8-FIX, providing the rationale for the following surveillance plan (~~Manno, 2006, Nature Med.).~~ Nathwani, 2011, Mol.Ther.). Each ~~patient~~subject will have a comprehensive surveillance plan ~~monitoring~~ that monitors LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a ~~patient's~~ subject's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. **Dose selection**: The benefit of this study can only be enhanced by selecting a starting dose that ~~potentially could contribute to a better~~ maximizes the opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and 6E13 vg/kg in the normal mouse, disease model mouse and monkey, respectively. Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to ~~patients but may potentially benefit the patient~~ subjects with the best chance of benefiting the subject therapeutically.	1, 5
9/Investigational Plan	Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled ~~sequentially~~ into one of up to three cohorts according to dose level: 1. 6E12 ~~vector genomes [~~vg~~] per kilogram~~/kg of body weight, given as a single intravenous dose (iv) 2. 2E13 vg ~~per kilogram~~/kg, iv 3. 6E13 vg ~~per kilogram~~/kg, iv ~~Patients will be enrolled sequentially every 3 weeks or more between cohorts. Dose escalation may occur after a single patient has been safely dosed if the resulting FVIII activity at Week 3 is < 5 IU/dL. Three weeks is expected to be the time the~~	5

Section No./Title	Text Revisions	Rationale
	~~expression will be close to the maximum. This escalation paradigm is intended to minimize the patient numbers exposed to subtherapeutic doses.~~ The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in ~~non-human primates~~ mice. ~~Approximately three weeks after an injection,~~ Cohorts will be enrolled sequentially, allowing a period of 3 weeks or more between cohorts. Dose escalation may occur after a single subject in a cohort has been safely dosed if the resulting FVIII activity at the Week 3 visit is < 5 IU/dL in both assays. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the subject numbers exposed to subtherapeutic doses. The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study. If the FVIII activity ~~is~~reaches ≥5 IU/dL at the Week 3 visit, then the ~~other patients of~~remaining subjects in the ~~dose group~~cohort will be enrolled without ~~waiting~~the need to wait for 3 weeks between ~~patients~~subjects. Subject 1 (Cohort 1) will be dosed by intravenous ~~perfusion~~ infusion with 6E12 ~~vector genomes [~~vg~~] per kilogram~~/kg of body weight. If the FVIII activity level does not reach ~~≥~~5 IU/dL or greater (expressed as % in Figure 9.1.1) at ~~21 days~~ the Week 3 visit in both assays, then ~~a higher~~ no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg ~~per kilogram) will be used for the next patient.~~ /kg). If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL ~~after Patient 2~~at the Week 3 visit in both assays, then ~~the highest dose (6E13 vg per kilogram)~~no further subjects will be ~~used for~~ dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next ~~patient.~~ Subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study. For the first subject treated in each ~~dose~~ Cohort, if the activity level reaches ≥5 IU/dL and ~~if~~ no safety issue is found, then up to four ~~patients will receive~~ subjects will be enrolled in the Cohort. Although the optimum design of this ~~dose. If at any time~~ study is three cohorts of 4 subjects, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels ~~reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be~~	

Section No./Title	Text Revisions	Rationale
	~~dosed at this dose level for a total of 6 patients per dose.~~ of FVIII and safety signals. Because ~~patients~~subjects develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a likely treatment option and these ~~patients~~subjects have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after ~~dose.~~ dosing. Any safety signal ~~will~~ may trigger a review of the data and possible additional ~~analysis including~~ immunogenicity studies that include an assessment of cellular immune responses using collected peripheral blood mononuclear ~~collection.~~ cells (PBMC). Table 9.1.4, dealing with steroid prophylaxis in the event of elevated LFTs, is discussed in Section 9.4.8.2.	
Table 9.1.1/Schedule of Events/Screening and Infusion/Assessment	Distribution of subject diaries and training in their use ~~Mononuclear cell~~ PBMC collection for CTL baseline Haemo-QoL-A Quality of Life (QoL) assessment	5
Table 9.1.1/Schedule of Events/Screening and Infusion/Rescreening	Added this new column	3
Table 9.1.1/Schedule of Events/Screening and Infusion/Footnotes	[f] Includes HLA ~~typing~~ genotyping, FVIII ~~mutation status~~ genotyping, TNFα and IL10a single nucleotide polymorphisms. [h] Should the screening visit occur within 7 days of the drug infusion, physical examination, ~~vital signs,~~ blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline. [i] Smart rescreening should only be performed if a patient has been determined to be eligible for the study and is unable to complete the Baseline assessments and Infusion prior to the closing of the original Screening window. Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once. [j] Only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification) assay must be done at smart rescreening. [k] Assessments on the day of infusion should be performed prior to the infusion. On the day of the BMN 270 Infusion, vital signs will be monitored prior to the infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5	3

Section No./Title	Text Revisions	Rationale
	minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals (± 15 minutes).	
Table 9.1.2/Schedule of Events/Post-Infusion Follow-Up/Assessment	Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) ~~hFVIII activity~~ FVIII assays[c] ~~hFVIII~~ FVIII antibody titer Haemo-QoL-A QoL assessment ~~Mononuclear cell~~ PBMC collection	5
Table 9.1.2/Schedule of Events/Post-Infusion Follow-Up/Follow-Up After BMN270 Administration - Weeks	Expanded Week 1 column to include D2, D4, and D8.	5
Table 9.1.2/Schedule of Events/Post-Infusion Follow-Up/Footnotes	[*] Visit windows are ±48 hours (and include the Day 4 visit) [c] Includes hFVIII activity level (APTT and FXa chromogenic assay), ~~hFVIII activity confirmation (frozen plasma),~~ Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. [d] Collection to occur on Day ~~1, 3, 7~~2 and 4 following BMN 270 infusion, and then weekly until at least 3 consecutive negative results are obtained.	1, 5
Table 9.1.3/Schedule of Events/Safety Follow-Up/Assessment	Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) ~~hFVIII activity~~ FVIII assays[c] ~~hFVIII~~ FVIII antibody titer ~~Mononuclear Cell~~ PBMC Collection (for determination of FVIII and Capsid specific CTL activity) Haemo-QoL-A QoL assessment	5
Table 9.1.3/Schedule of Events/Safety Follow-	[*] Visit windows are ~~±72 hours~~1 week for visits in Year 1, and ± 2 weeks for visits in Years 2-5.	5

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Up/Footnotes	[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), ~~hFVIII activity confirmation (frozen plasma),~~ Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72 hour interval without FVIII use is achieved. [e] Haemo-QoL-A QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).	
Table 9.1.4/Schedule of Events/Steroid Prophylaxis	This table was added as part of this amendment.	1
9.2/Discussion of Study Design, Including Choice of Control Group	The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and ~~a panel of investigators participating in the study.~~ the DRB. There will be no control group. Parameters for each ~~patient~~subject will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).	5
9.3/Selection of Study Population	Up to 12 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation. ~~A total of 6 patients may be enrolled at a single dose level.~~	5
9.3.1/Inclusion Criteria	• Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their base FVIII ~~baseline~~ level is 1 IU/dL or less • Treated/exposed to FVIII concentrates or ~~cryoprecipitates~~ cryoprecipitate for a minimum of 150 exposure days (EDs) • No history of inhibitor, ~~or no inhibitor on 2 consecutive occasions within the past 12 months using~~ and results from a modified Nijmegen Bethesda assay ~~higher~~ of less than 0.6 Bethesda ~~Unit~~ Units (BU) 2 consecutive occasions at least one week apart within the past 12 months	5
9.3.1/Exclusion Criteria	• Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition ~~and~~ or AAV5 total antibodies • ~~Patients that are~~ HIV positive ~~are excluded.~~ • Significant liver dysfunction as defined by abnormal elevation of:	1, 2, 5

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	○ ALT (alanine transaminase) to 3 times the upper limit of normal~~, bilirubin, ~~; ○ Bilirubin above 3 times the upper limit of normal; ○ Alkaline phosphatase~~,~~ above 3 times the upper limit of normal; or ○ ~~an~~ INR (international normalized ratio) ~~of~~ ≥ 1.4. • Treatment with any IP within 30 days prior to the end of the screening ~~visit~~ period • Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study~~.~~ including possible corticosteroid treatment outlined in the protocol. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion. • ~~Receipt of~~ Prior treatment with any ~~vector or~~ gene transfer agent • Major surgery planned in the 16-week period following the viral infusion • Use of immunosuppressive agents or live vaccines within 30 days before the viral infusion	
9.3.3.1/Study Safety Evaluation Criteria	If any of the following events occur ~~(except the persistence of AAV5)~~ in a ~~patient~~subject in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1. • A 5-fold increase from baseline in ALT after BMN 270 administration • The occurrence of ~~other drug~~ Grade 3 or higher adverse events assessed as related ~~Grade III-IV toxicity~~ to study drug, including liver failure and clinical hepatitis • ~~Drug~~ Grade 2 adverse event assessed as related ~~Grade II toxicity~~ to study drug that persists for at least 7 days • The ~~occurrence~~ detection of neutralizing antibodies to hFVIII following BMN 270 infusion The occurrence of a malignancy excluding skin cancers at any point after BMN 270 infusion~~ that is assessed as possibly, probably, or definitely related to the study agent~~	5
9.3.5/Subject Identification and Replacement of Subjects	~~Patients~~ Subjects who withdraw from the study ~~after Week 16~~ will not be replaced.	5
9.4.2.1/Product Characteristics and	BMN 270 is a sterile, clear, colorless-to-pale yellow solution for IV infusion and is supplied in a 2 mL polypropylene cryovial.	5

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Labeling	Each vial contains 1.1 mL of AAV5-hFVIII-SQ at a concentration of 2E13 vector genomes per mL in a pH 7.4 phosphate buffer.	
9.4.4/Directions for Administration	FVIII ~~protein concentrate~~ replacement therapy will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions. BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe pump will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump at a constant rate of 4 ml/min while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours~~.~~, although the IP has been shown to be stable at room temperature for 6 hours. ~~Patients~~ Subjects will remain ~~hospitalized~~ in the clinic for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours ~~of hospitalization~~, participants will be discharged from the clinic unless toxicity has been observed in which case ~~hospitalization~~ the stay in the clinic may be extended or the subject may transfer to ~~an outpatient~~ a separate facility ~~may occur~~ based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.	5
9.4.5/Method of Assigning Subjects to Treatment Groups	~~Patients~~ Subjects who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study ~~patient~~subject. Upon their enrollment into the study, ~~patients~~subjects will be assigned a unique ~~patient~~subject number and dose level by the Sponsor. ~~Patients~~Cohorts are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. ~~Patients~~Cohorts may receive the next higher dose if subjects in the previous ~~patient~~ cohort does not meet the activity criteria, or the same dose if subjects in the previous ~~patient~~ cohort meets the activity criteria. ~~Up to 6 patients can have the same dose, for a total of 12 patients in this study.~~	5
9.4.6/Selection of Doses Used in the Study	The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in ~~non-human primates.~~ Mice. The dose escalation is half-logs based.	5
9.4.6.2/Selection of Dose for Each Subject	The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus potentially decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).	5

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	Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and ~~a panel of investigators participating in the study~~ the DRB. ~~Patient~~ Subject 1 (Cohort 1) will be dosed by intravenous infusion with 6E12 ~~vector genomes [~~vg~~] per kilogram~~/kg of body weight. If the FVIII activity level does not reach ≥5 IU/dL or greater (expressed as % in Figure 9.1.1) at ~~21 days~~ the Week 3 visit in both assays, then ~~a higher~~ no further subjects will be dosed in Cohort 1 and enrollment into Cohort 2 will initiate with Subject 2 at the next dose (2E13 vg ~~per kilogram) will be used for the next patient.~~ /kg). If the FVIII activity level in the first subject treated in Cohort 2 does not reach ≥5 IU/dL ~~after Patient 2~~at the Week 3 visit in both assays, then ~~the highest dose (6E13 vg per kilogram)~~no further subjects will be ~~used for~~ dosed in Cohort 2 and enrollment into Cohort 3 (6E13) will initiate with the next ~~patient.~~ Subject. If the FVIII activity level in the first subject treated in Cohort 3 does not reach ≥ 5 IU/dL at the Week 3 visit in both assays, then the DRB will recommend whether to continue to add subjects to the cohort and/or whether to continue the study. For the first subject treated in each ~~dose~~ Cohort, if the activity level reaches ≥5 IU/dL and ~~if~~ no safety issue is found, then up to four ~~patients will receive~~ subjects will be enrolled in the Cohort. Although the optimum design of this ~~dose. If at any time~~ study is three cohorts of 4 subjects, the adaptive nature of this trial allows the DRB to recommend allocating subjects to the cohorts based on activity levels ~~reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose~~ of FVIII and safety signals.	
9.4.8/Prior and Concomitant Medications	The following medications are prohibited starting 30 days before Screening: • Systemic immunosuppressive agents	5
9.4.8.2/Glucocorticoid Treatment of Elevated Hepatic Transaminases	Reports of abnormal LFTs (defined as 1.5x the ~~patient's~~ subject's baseline ALT level) should be made to the BioMarin medical monitor within ~~24 hours~~30 minutes of the lab value being available~~. If the LFTs are abnormal, ~~, and treatment with prednisolone will be initiated immediately at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first ~~week~~2 weeks, then 40 mg the ~~second week~~ next 2 weeks, then 30 mg the ~~third week~~ next two weeks, then 20 mg the ~~fourth week~~ next two weeks, then stop~~) will be ~~, for a total treatment of 8 weeks). Local laboratory results of LFTs will be used to trigger corticosteroid treatment as needed. In case of discrepant results between local and central laboratories, the Investigator and Medical Monitor will decide further action.	1, 4

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	If a glucocorticoid treatment is started for a subject at any point of the study, the following steps will be taken: • A prophylactic glucocorticoid treatment will be started for subsequent subjects scheduled to be dosed. Subjects will receive prednisolone 40 mg per day starting at week 3, for a duration of 4 weeks, then tapered by 5 mg every two weeks for 8 weeks (until 20 mg/day is reached), then tapered 5 mg per week for 3 weeks, for a total duration of 15 weeks (refer to Table 9.1.4~~immediately~~). o Subjects already dosed who have not yet reached Week 17 in the study will be evaluated for the use of prophylactic steroids after discussion between the Investigator and the Medical Monitor • Once prophylactic glucocorticoid treatments have been initiated, then all subjects (who have not already passed that period in the study) will have 3x/week LFT assessments during the 6-week period when a liver response is expected based on the liver response in the original subject, if this period is different from the 3x/weekly LFT assessments already planned for Weeks 7-12. o Subjects who have already passed that 6-week period in the study will continue to have the regularly scheduled LFT assessments as planned in the protocol. Management and monitoring of reactions to corticosteroids should be determined by the Investigator's clinical judgment in consultation with the Sponsor's Medical Monitor. This includes the contraindicated use of NSAIDs during corticosteroid treatment, and specific monitoring not already covered by the schedule of events. Hepatitis B status will be rechecked at the end of the corticosteroid treatment. All adverse events should be reported as outlined in section 10 of the protocol.	
9.4.9/Treatment Compliance	Study drug will be administered to ~~patients~~ subjects at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return or destroy all used and unused study drug containers.	5
9.5/Investigational Product Accountability	The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received~~, patients to whom IP is dispensed (patient-by-patient dose-specific accounting),~~ and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.	5
9.7.2/Primary Efficacy	The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy	5

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Variables	goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to stable expression of FVIII at 5 IU/dL or above, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes (~~Nathwani, 1992, Baillieres Clin.Haematol.).~~Den Uijl, 2011, Haemophilia). If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. The FVIII activity level in both assays and the number of ~~patients~~subjects with FVIII activity ≥ 5 IU/dL in at least one of the two assays will be summarized.	
9.7.3/Secondary Efficacy Variables	~~Assessment of viral shedding at one week after BMN 270 infusion will also be considered an efficacy variable.~~	5
9.7.4/Immunogenicity	Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of ~~6~~5-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to ~~an~~a retrospective immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.	5
9.7.6/Exploratory Biomarker Assessments	All biomarker samples collected in this study may be used for exploratory biomarker research including evaluation of additional biomarkers not specifically listed in the protocol. In addition, samples collected for other purposes may be used for exploratory use once the primary use has been completed. No additional genetic or genomic testing will be conducted on any sample (other than testing specified in the protocol).	5
9.7.7/Haemo-QoL-A Quality of Life Assessment	The Haemo-QoL-A is a patient-reported outcome (PRO) questionnaire which will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.	5
Table 9.7.8.2.1/Clinical Laboratory Tests/Footnotes	ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; ~~T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone;~~ WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.	5

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9.7.8.3/Liver Function and Hepatitis Testing at Screening	~~Patients~~ Subjects will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. ~~If medical records~~ Subjects with documented results showing ~~no evidence~~ an absence of active Hepatitis B or Hepatitis C infection ~~from the previous 30 days are available, then the Screening tests do not need to be done.~~ ~~Evidence of ongoing infection (~~(as measured by positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) ~~will be an exclusion criteria. If these parameters are negative and therefore no active infection is detected, then the patients may be included if they were vaccinated (for hepatitis B) or cleared the infection (for hepatitis C) and their liver function is acceptable.~~30 days prior to providing signed informed consent do not need to be screened again. Evidence of ongoing Hepatitis B or Hepatitis C infection is exclusionary. Subjects who have cleared a Hepatitis B infection or are seronegative do not need to receive the Hepatitis B vaccination. A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as: • More than 3x the normal Alkaline phosphatase ~~above the normal cut off~~ level. • INR ~~over~~≥ 1.4. Liver ultrasound results indicative of a liver cirrhosis	1, 4
9.7.8.4/Vital Signs, Physical Examinations and Other Observations Related to Safety	Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored prior to infusion, during the infusion every 15 minutes (± 5 minutes), following the infusion hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals~~.~~ (± 15 minutes). A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems ~~(level of consciousness, speech, language, cranial nerves, motor strength, motor tone, abnormal movements, reflexes, upper extremity sensation, lower extremity sensations, gait, Romberg, nystagmus, and coordination).~~	5
9.7.8.5/Viral Shedding	Viral shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Eng.J.Med (Supplemental Appendix); Manno, 2006, Nature Med. (Supplemental Appendix); Schenk-Braat, 2007, J Gene Med; Croteau, 2004, Ann Occup Hyg). ~~It has been constantly shown that~~ In the literature referenced above, vector was ~~not~~ no longer detectable	5

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	~~anymore at~~ after 40 days~~, with its persistence~~ in blood, saliva, urine or stool. However, vector persisted in semen for 16 weeks in the seminal fluid but not in motile sperm (Manno, 2006, Nature Med.). In ~~addition, it has always~~ all cases, the amount of vector present in these bodily fluids involved ~~only~~ an extremely small fraction of the ~~injected~~ infused dose. Viral shedding will also be extensively studied in the present clinical trial, every week up to 16 weeks, then every 4 weeks to one year, then every three months~~.~~, until at least 3 consecutive negative results are obtained. During the Post-Infusion Follow-Up period, ~~patients~~ subjects will undergo testing of various bodily ~~fluids~~ samples to look for evidence of viral shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2 and Table 9.1.3.	
10.1.1/Adverse Events	Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of major trauma, should be recorded as adverse events.	5
10.2/Serious Adverse Events	A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria: • Requires or prolongs inpatient hospitalization. ~~Hospitalization for less than 24 hours will not be considered to be an SAE. Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.~~	5
10.2.1/Events of Special Interest (EOSI)	The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality: Elevation of liver enzymes ~~triggering~~ (ALT) that triggers a corticosteroid treatment	5
10.3.3/Assessment of Seriousness, Severity, and Causality	The Investigator responsible for the care of the ~~patient~~subject or medically qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments ~~should~~ must be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).	5
10.4.1.7/Hospitalization, Prolonged Hospitalization, or Surgery	There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to: • Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.	5

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10.4.1.9/Pregnancy	Pregnancy in partner should be reported within 24 hours of the site becoming aware of the pregnancy by ~~faxing~~ entering the information on the Pregnancy ~~Form in the study reference materials~~ eCRF and submitting to BPV~~.~~ within 24 hours of the site becoming aware of the event. The Investigator must make every effort to follow the ~~patient~~ subject's partner through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study ~~patient~~subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.	5
10.5.1/Expedited Reporting Requirements	~~The reporting period for SAEs begins after informed consent is obtained and the first administration of study drug is given. It continues for approximately 5 years or until study discontinuation/termination, whichever is longer.~~	5
12.2/Screening Visit	The following procedures will be performed during the Screening Period: • Full medical history, including haemophilia A history, Hepatitis B, Hepatitis C, and HIV. ~~Patients with documented negative results within the last 30 days do not need to be retested.~~ • Samples for hFVIII Assays ○ AAV5 transduction inhibition ~~assays~~ assay • Screen for Hepatitis B, Hepatitis C, and HIV if required (~~Patients~~subjects with documented negative results ~~within the last~~ 30 days prior to informed consent being obtained do not need to be retested) • Blood samples for Biomarker testing (including HLA ~~typing~~ genotyping, FVIII ~~mutation~~ genotyping status, TNFα and IL10a single nucleotide polymorphisms)	4, 5
12.2.1/"Smart Rescreening" Visit	Subjects who undergo smart rescreening must complete the rescreening assessments and receive the infusion within 90 days of signing the original consent. Subjects who do not complete dosing within 90 days will be required to re-consent and undergo all screening procedures. Subjects may not undergo smart rescreening more than once. If a patient has to be screened again because the original assessments have fallen out of the 28 + 14 day period allowed for Screening (refer to Section 12.2), then only the following assessments need to be performed (rather than the full list indicated in Section 12.2) for the patient to be successfully re-screened for the study: • Vital signs	3, 5

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	• Assessment of Adverse Events and Concomitant Medications
	• Documentation of bleeding episodes and FVIII usage (by either subject or clinical information)
	• hFVIII Assays (only the hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
	• Blood sample for AAV5 Assays
	  ○ AAV5 antibody titer
	  ○ AAV5 transduction inhibition assay
	Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)	

12.3/Baseline Visit	Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:
	• ~~Samples for FVIII Assays~~
	  ~~○ FVIII activity chromogenic FXa (plasma)~~
	  ~~○ FVIII activity level APTT One stage assay~~
	  ~~○ FVIII coagulation activity exploratory assay~~
	  ~~○ FVIII inhibitor level (Bethesda assay with Nijmegen modification)~~
	  ~~○ FVIII antibody assay~~
	  ~~○ FVIII antigen (ELISA)~~
	• Distribution of subject diaries and training in diary completion
	• ~~Mononuclear cell~~ PBMC collection for CTL baseline
	• Haemo-QoL-A QoL assessment
		5

12.4/Treatment Visit/BMN 270 Infusion Visit (Day 1)	There will be one treatment visit for each ~~patient. Patients~~ subject. Subjects will ~~be hospitalized~~ remain in the clinic for 24 hours for the BMN 270 Infusion Visit.	5

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12.5.1/Every Visit	Every week (Weeks 1 through 16), the following procedures will be performed: • Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) • Samples for FVIII Assays ~~o FVIII activity confirmation (Frozen plasma)~~ Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. • PCR of vector genomes in blood, saliva, urine, semen, and stools o Collection to occur ~~on Day 1, 3, 7 following BMN 270 infusion, and then~~ weekly until at least 3 consecutive negative results are obtained	1, 5
12.5.3/Week 1 – Day 2 and Day 4	On Day 2 and Day 4 of Week 1, the following procedures will be performed: • PCR of vector genomes in blood, saliva, urine, semen, and stools (Day 2 and Day 4) • Samples for FVIII Assays (Day 4 only) o FVIII activity level (APTT) o FVIII activity level (chromogenic FXa assay) o FVIII coagulation activity exploratory assay o Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level o FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved.	5
12.5.4/Every 2 Weeks	Every 42 weeks (Weeks 2, 4, 6, 8, 10, 12, 14 and 16), the following procedure will be performed: • ~~Mononuclear cell~~ Exploratory biomarker assessments	5
12.5.5/Weeks 4, 8, 12,	At Weeks 4, 8, 12, and 16, the following procedure will be performed:	5

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and 16	• PBMC collection	
12.5.7/Weeks 8 and 16	At Weeks ~~1, 8,~~ and 16, the following procedure will be performed: • AAV5 antibody titer	5
12.5.8/Weeks 1, 2, 3, 4, and 16	At Weeks 1, 2, 3, 4, and 16, the following procedure will be performed: • Haemo-QoL-A QoL assessment	5
12.6/Safety Follow-Up – Year 1	After the 16 weekly infusion follow-up visits are complete, subjects will return to the study site at Weeks 20, 28, 36, 44, and 52 (± ~~48 hours~~1 week), when the following procedures will be completed:	5
12.6.1/Every Visit	At Weeks 20, 28, 36, 44, and 52, the following procedures will be performed: • Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) • FVIII Assays o ~~FVIII activity confirmation (Frozen plasma)~~ Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. • ~~Mononuclear cell~~ PBMC collection • PCR of vector genomes in blood, saliva, urine, semen, and stools o Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.	5
12.6.2/Weeks 28 and 52	At Weeks 28 and 52, the following procedure will be performed: • Haemo-QoL-A QoL assessment	5
12.7/Safety Follow-Up	During Years 2-5 of Safety Follow-up, ~~patients~~ subjects will be assessed every 3 months (± ~~72 hours~~2 weeks). At these times, the following procedures will be completed:	5
12.7.1/Every Visit	Every 3 months (± ~~72 hours~~ 2 weeks), the following procedures will be performed:	5

Section No./Title	Text Revisions	Rationale
	• Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) • FVIII Assays o ~~FVIII activity confirmation (Frozen plasma)~~ Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. • ~~Mononuclear cell~~ PBMC collection • PCR of vector genomes in blood, saliva, urine, semen, and stools o Sample testing during Safety Follow-Up is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period.	
12.7.2/Every Other Visit (Every 6 months)	Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedure will be performed: • Haemo-QoL-A QOL assessment	5
12.8/Early Termination Visit	If a ~~patient~~ subject leaves the study prior to the Week ~~270~~260 visit, the ~~patient~~ subject will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done: • Assessment of Adverse Events and Concomitant Medications (including review of subject diary for bleeding and FVIII use) o Bethesda assay (with Nijmegen modification) for FVIII inhibitor level o ~~FVIII activity confirmation (Frozen plasma)~~ o FVIII antigen (ELISA) Note: If a subject has used FVIII within 72 hours of a measurement day, all efforts should be made to obtain FVIII activity measurements when a 72-hour interval without FVIII use is achieved. • ~~Mononuclear cell~~ PBMC collection • PCR of vector genomes in blood, saliva, urine, semen, and stools	5

Section No./Title	Text Revisions	Rationale
	○ Sample testing at the ETV is not required if at least 3 consecutive samples are clear during the Post-Infusion Follow-Up period. • Haemo-QoL-A QOL assessment	
12.9/End of Study	The study will end after the last ~~patient~~subject completes the last Safety Follow-Up visit (Week ~~270~~260).	5
13/Data Quality Assurance	BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, ~~CRFs~~ eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs. At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on ~~CRFs~~ eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records. ~~The designated clinical data management group will enter or transfer CRF data into a study database.~~ Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.	5
14.2/Primary and Secondary Efficacy Analysis	Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay ~~between 1 to~~ at 16 weeks following study treatment.	5
14.3/Immunogenicity	Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons ~~across doses.~~	5
14.6/Determination of Sample Size	No formal sample size calculations based on statistical power were performed. The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 12 ~~patients~~subjects may be dosed in the study; the actual number of ~~patients~~subjects will depend on the criteria for dose escalation. ~~A total of 6 patients may be enrolled at a single dose level.~~	5
15/Data Review Board	**DATA ~~MONITORING COMMITTEE~~REVIEW BOARD**	5
21/References	Den Uijl IEM, Mauser Bunschoten EP, Roosendaal EG, Schutgens REG et al. Clinical severity of haemophilia A: does the	5

Section No./Title	Text Revisions	Rationale
	classification of the 1950s still stand? Haemophilia. 2001; 17(6):849-853. Hay CRM, DiMichele DM. The principal results of the International Immune Tolerance Study: A randomized dose comparison. Blood 2012; 119 [6]:1335-1344. Kaufman RJ, Powell JS. Molecular approaches for improved clotting factors for hemophilia. Blood 2013; 122: 3568–74. Mannucci PM & Tuddenham EGD. The Hemophilias - From Royal Genes to Gene Therapy. NEJM 2001; 344:1773-1779. Nathwani AC, Gray JT, Ng CY, et al. Self-complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. Blood. 2006; 107(7):2653-2661.	

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Patient Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015
Date of Amendment 2:	26 May 2015

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 2

Date: 26 May 2015

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 2 to the BMN 270-201 protocol is provided below.

1. Exclusion criterion changed from "Evidence of detectable viral load of HIV" to "Patients that are HIV positive are excluded." In addition, the following exclusion criterion was added: "Any evidence of active infection or any immunosuppressive disorder."

Rationale: The criteria were amended to exclude patients with any disease or condition that may jeopardise patient safety.

2. The Overall Study Design and Plan changed to include details of Sponsor actions should any of the criteria listed in Section 9.3.3.1 occur.

Rationale: Sponsor actions pertaining to the criteria listed in Section 9.3.3.1 reflect a more conservative approach considering this is a first-in-man trial.

3. Expedited Reporting Requirements have been updated to include SUSAR reporting in accordance with European Directive 2001/20/EC.

Rationale: The protocol was amended to include the Sponsor's responsibility to ensure that all relevant information about SUSARs is reported to the competent authorities in all the Member States concerned, and to the Ethics Committee in compliance with current legislation.

4. Removed "Exploratory FVIII activity assay" from Primary Efficacy Variables section.

Rationale: The Exploratory FVIII activity assay is currently used for exploratory purposes only and not to direct patient management.

5. Expanded description of Data Review Board (DRB) in Section 15 to include notification of all DRB meetings and meeting outcomes to participating sites.

Rationale: In view of the multiple sites involved, the DRB meetings and meeting outcomes will be used to disseminate data (safety and efficacy) to participating sites.

6. Removed requirement of FVIII Treatment Washout period.

Rationale: While washout periods are used in some Haemophilia studies, after discussion with Investigators and Experts, it was recommended to remove the washout period. Removing the washout period allows for continued prophylactic treatment up to the day of the infusion and may reduce the risk of a bleeding episode prior to BMN 270 treatment.

Specific changes included in this amendment, including the Synopsis, since Amendment 1 (approved 06 March 2015) are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for rhFVIII (under 24 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis up to 30 to 50 for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and

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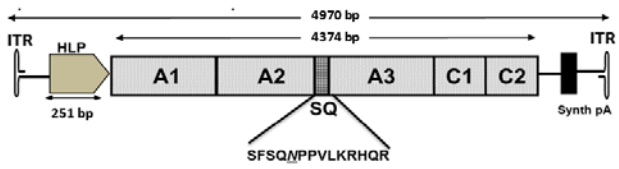
thus, show promise in reduced dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 1).

Figure 1. BMN 270 transgene construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector

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dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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 expression of 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

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STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled sequentially into one of up to three cohorts according to dose level:

1. 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
2. 2E13 vg per kilogram, iv
3. 6E13 vg per kilogram, iv

Patients will be enrolled sequentially every 3 weeks or more between cohorts. Dose escalation may occur after a single patient has been safely dosed if the resulting FVIII activity at Week 3 is < 5 IU/dL. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the patient numbers exposed to subtherapeutic doses.

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity is ≥ 5 IU/dL, then the other patients of the dose group will be enrolled without waiting for 3 weeks between patients.

Patient 1 will be dosed by intravenous perfusion with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥ 5 IU/dL at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥ 5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and no safety issue is found, then up to four

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patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Because patients develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a treatment option and these patients have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dose. Any safety signal will trigger a review of the data and possible additional analysis including mononuclear collection.

NUMBER OF PATIENTS PLANNED:

Up to 12 patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII baseline level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitates for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, or no inhibitor on 2 consecutive occasions within the past 12 months using a modified Nijmegen Bethesda assay higher than 0.6 Bethesda Unit (BU)
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition and AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.

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3. Patients that are HIV positive are excluded.
4. Significant liver dysfunction as defined by abnormal elevation of ALT (alanine transaminase) to 3 times the upper limit of normal, bilirubin, alkaline phosphatase, or an INR (international normalized ratio) of 1.4. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
5. Evidence of any bleeding disorder not related to Haemophilia A
6. Platelet count of $< 100 \times 10^9$/L
7. Creatinine $\geq$ 1.5 mg/dL
8. Liver cirrhosis of any etiology as assessed by liver ultrasound
9. Hepatitis B if surface antigen is positive
10. Hepatitis C if RNA is positive
11. Treatment with any IP within 30 days prior to the screening visit
12. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
13. Receipt of any vector or gene transfer agent

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each patient will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous perfusion.

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CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver function tests (LFT)

No major toxicity is expected based on preclinical studies in mice and monkeys. An asymptomatic transaminitis was observed at week 8-10 after administration with an AAV8-FIX, providing the rationale for the following surveillance plan. Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.

There will be a detailed monitoring plan for assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Patients will be asked to keep a patient diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be

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used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 12 patients may be dosed in the study. The patients will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at $\geq$ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay between 1 to 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as viral shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
eCRF	electronic case report form
DMC	Data Monitoring Committee
DRB	Data Review Board
ED	exposure days
EOSI	events of special interest
ETV	early termination visit
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
HA	Haemophilia A
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6

IEC	independent ethics committee
IND	Investigational New Drug (application)
INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LFT	liver funtion test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
vg	vector genomes

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the patients, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all patient recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible patients for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the patients will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each patient will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Patient Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each patient prior to entering the patient into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF , and if applicable, a copy of the approved patient information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each patient and will maintain the original in the record file of the patient.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to patients. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of patients in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each patient as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Laboratory evaluations will be performed at the local laboratories associated with the study sites. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to around 20 hours (Pipe, 2010, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration or problems of FVIII inhibitor formation, which occurs in 30% of patients on standard FVIII replacement therapy (Nathwani, 1992, Baillieres Clin.Haematol.).

Gene therapy offers the potential of a cure through continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2012, Haemophilia); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Paneda, 2009, Hum.Gene Ther.). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents > 30-fold safety factor from the no observed adverse effect level (NOAEL) in enabling nonclinical studies.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Eng.J.Med.; Nathwani, 2014, N.Eng.J.Med.). The large size of the FVIII cDNA was shortened and a

preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is also used in clinical trials and has shown good safety in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola. 2014, J Hepatology).

AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin. Haematol). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. . The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for rhFVIII (under 24 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis up to 30 to 50 for on demand treatment) (Nagel, 2011, Haemophilia). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need

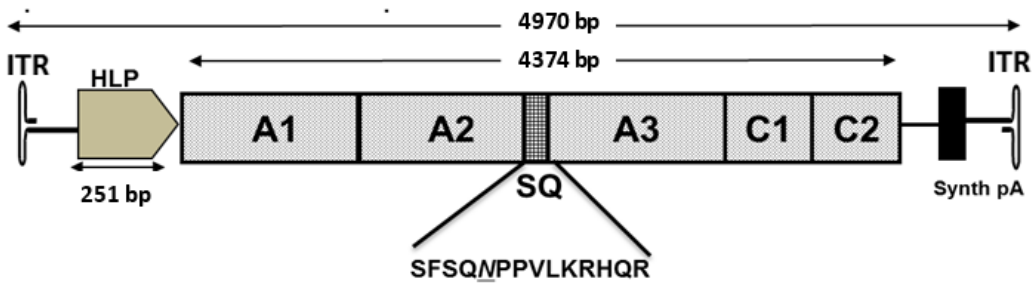
for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Pipe, 2010, Blood). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector(Nathwani, 2011, NEJM). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.). (Bainbridge, 2008, NEJM; Maguire, 2009, Lancet; Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: BMN 270 Transgene Construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 8-10 after administration with an AAV8-FIX, providing the rationale for the following surveillance plan (Manno, 2006, Nature Med.). Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that potentially could contribute to a better opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg.

A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to patients but may potentially benefit the patient therapeutically.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled sequentially into one of up to three cohorts according to dose level:

1. 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
2. 2E13 vg per kilogram, iv
3. 6E13 vg per kilogram, iv

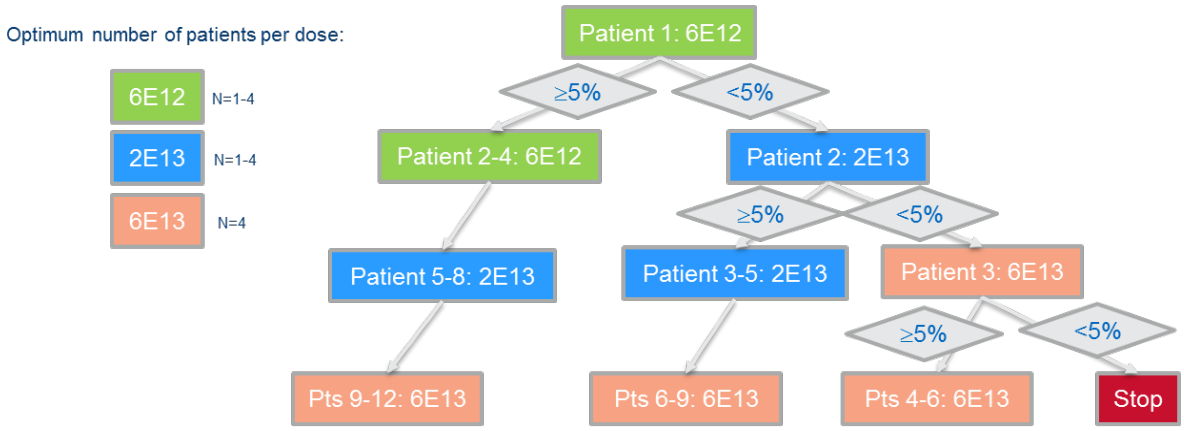
Patients will be enrolled sequentially every 3 weeks or more between cohorts. Dose escalation may occur after a single patient has been safely dosed if the resulting FVIII activity at Week 3 is < 5 IU/dL. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the patient numbers exposed to subtherapeutic doses.

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity is ≥5 IU/dL, then the other patients of the dose group will be enrolled without waiting for 3 weeks between patients.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme



Patient 1 will be dosed by intravenous perfusion with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥5 IU/dL (expressed as % in Figure 9.1.1) at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and if no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Because patients develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a treatment option and these patients have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dose. Any safety signal will trigger a review of the data and possible additional analysis including mononuclear collection. Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the Data Review Board (DRB) and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 for Safety Follow-up.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion		BMN 270 Infusion Visit (Day 1)
	Screening (Day -28 to Day -1)	**Baseline (Day -7 to Day -1)[h]**	
Informed consent	X		
Medical History	X		
Physical Exam[a]	X	X	X
Vital Signs	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X
Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)	X	X	
Electrocardiogram	X		
Chest X-ray	X		
Liver Ultrasound	X		
hFVIII Assays[b]	X	X	
AAV5 Assays[c]	X		X
Screen for Hepatitis B, Hepatitis C, HIV[d]	X		
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[e]	X	X	
Mononuclear cell collection for CTL baseline		X	
PCR of vector genomes in blood, saliva, urine, semen, and stools		X	
Biomarker testing[f]	X		
Exploratory biomarker assessments[g]		X	
Quality of Life (QoL) assessment		X	
BMN 270 Infusion			X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height and weight will be recorded at Screening only.

[b] Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA).

[c] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). The assessment on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[d] Patients with documented negative results within the last 30 days do not need to be retested.

[e] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[f] Includes HLA typing, FVIII mutation status, TNFα and IL10a single nucleotide polymorphisms.

[g] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

[h] Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up

	Follow-Up After BMN 270 Administration - Weeks*															
Assessment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical exam[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
hFVIII activity assays[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
hFVIII antibody titer	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Exploratory biomarker assessments[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
QoL assessment	X	X	X	X												X
AAV5 antibody titer	X							X								X
Testing for reactivation of hepatitis B and hepatitis C	X		X			X										
Mononuclear cell collection				X				X				X				X

* Visit windows are ±48 hours

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included. Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

[c] Includes hFVIII activity level (APTT and FXa chromogenic assay), hFVIII activity confirmation (frozen plasma), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA).

[d] Collection to occur on Day 1, 3, 7 following BMN 270 infusion, and then weekly until 3 consecutive negative results are obtained.

[e] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

Table 9.1.3: Schedule of Events – Safety Follow-Up

Assessment	Year 1 - Weeks*					Years 2-5*	ETV (early termination visit)
	20	28	36	44	52	Q3M	
Physical exam[a]	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]	X	X	X	X	X	X	X
hFVIII activity assays[c]	X	X	X	X	X	X	X
AAV5 antibody titer	X	X	X	X	X	X	X
hFVIII antibody titer	X	X	X	X	X	X	X
Mononuclear Cell Collection (for determination of FVIII and Capsid specific CTL activity)	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X
QoL assessment		X			X	X[e]	X

* Visit windows are ±72 hours

[a] Complete physical examination should be performed at Weeks 52 and every 52 weeks thereafter; brief physical exam may be performed at other study visits. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), hFVIII activity confirmation (frozen plasma), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA).

[d] Sample testing during Safety Follow-Up is not required if 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[e] QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Three doses of BMN 270 will be evaluated and the dose escalation decision tree is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

There will be no control group. Parameters for each patient will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 12 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII baseline level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitates for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, or no inhibitor on 2 consecutive occasions within the past 12 months using a modified Nijmegen Bethesda assay higher than 0.6 Bethesda Unit (BU)

6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition and AAV5 total antibodies
2. Any evidence of active infection or any immunosuppressive disorder.
3. Patients that are HIV positive are excluded.
4. Significant liver dysfunction as defined by abnormal elevation of ALT (alanine transaminase) to 3 times the upper limit of normal, bilirubin, alkaline phosphatase, or an INR (international normalized ratio) of 1.4. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
5. Evidence of any bleeding disorder not related to Haemophilia A
6. Platelet count of $< 100 \times 10^9$/L
7. Creatinine ≥ 1.5 mg/dL
8. Liver cirrhosis of any etiology as assessed by liver ultrasound
9. Hepatitis B if surface antigen is positive
10. Hepatitis C if RNA is positive
11. Treatment with any IP within 30 days prior to the screening visit
12. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
13. Receipt of any vector or gene transfer agent

9.3.3 Removal of Patients from Treatment or Assessment

Patients may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any patient who requests to be withdrawn. A patient's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all patient withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a patient from the study include, but are not limited to, the following:

- Patient requires medication or medical procedure prohibited by the protocol
- Patient does not adhere to study requirements specified in the protocol
- Patient was erroneously admitted into the study or does not meet entry criteria
- Patient is lost to follow-up

If a patient fails to return for scheduled visits, a documented effort must be made to determine the reason. If the patient cannot be reached by telephone, a certified letter should be sent to the patient requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each patient, before enrollment into the study, that for evaluation of study results, the patient's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each patient. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the patient and the patient will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur (except the persistence of AAV5) in a patient in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis per Section 9.1.

- A 5-fold increase in ALT after BMN 270 administration
- The occurrence of other drug-related Grade III-IV toxicity, including liver failure and clinical hepatitis
- Drug-related Grade II toxicity that persists for at least 7 days
- The occurrence of neutralizing antibodies to hFVIII following BMN 270 infusion

- The persistent detection of the AAV vector genome in the semen of a participant more than 26 weeks after BMN 270 infusion, as discussed in Section 9.7.8.5
- The occurrence of a malignancy at any point after BMN 270 infusion that is assessed as possibly, probably, or definitely related to the study agent
- Any unexplained serious adverse event ≥ grade 4 assessed as at least possibly related to study drug

9.3.4 Patient Identification and Replacement of Patients

Each patient will be assigned a unique patient identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Patients who withdraw from the study after Week 16 will not be replaced.

9.3.5 Duration of Patient Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be

stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical exam performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (e.g. the median cubital vein) and flushed with saline. FVIII protein concentrate will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Patients will remain hospitalized for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours of hospitalization, participants will be discharged unless toxicity has been observed in which case hospitalization may be extended or transfer to an outpatient facility may occur based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Patients to Treatment Groups

Patients who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study patient. Upon their enrollment into the study, patients will be assigned a unique patient number and dose level by the Sponsor.

Patients are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Patients may receive the next higher dose if the previous patient does not meet the activity criteria, or the same dose if the previous

patient meets the activity criteria. Up to 6 patients can have the same dose, for a total of 12 patients in this study.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in non-human primates. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Patient

A minimum of three weeks are required between patients, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next patient will be made as described below.

9.4.6.2 Selection of Dose for Each Patient

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

Patient 1 will be dosed with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥5 IU/dL at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and if no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Refer to Figure 9.1.1 for a visual representation of the study design.

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications taken by a patient for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Immunosuppressive agents

9.4.8.1 Concomitant Haemophilia Treatments

Patients on "on demand" therapy for FVIII will continue their treatment at will. Patients on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the patient, who will carefully record his treatment and bleeding episodes in his diary.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period, each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

Reports of abnormal LFTs (defined as 1.5x the patient's baseline level) should be made to BioMarin within 24 hours of the lab value being available. If the LFTs are abnormal, treatment with prednisolone initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first week, then 40 mg the second week, then 30 mg the third week, then 20 mg the fourth week, then stop) will be started immediately.

9.4.9 Treatment Compliance

Study drug will be administered to patients at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received, patients to whom IP is dispensed (patient-by-patient dose specific accounting), and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to stable expression of FVIII at 5 IU/dL, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes (Nathwani, 1992, Baillieres Clin.Haematol.).

To measure the primary efficacy variable, the following assays will be used:

- FVIII activity (chromogenic FXa assay)
- FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time)

The FVIII activity level and the number of patients with FVIII activity ≥ 5 IU/dL will be summarized.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Patients will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the patient's diary or other patient records.

Assessment of viral shedding at one week after BMN 270 infusion will also be considered an efficacy variable.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 6-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to an immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage APTT and/or chromogenic FXa assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2 and Table 9.1.3.

9.7.6 Exploratory Biomarker Assessments

Blood samples will be collected from subjects at the time points indicated in Table 9.1.1 and Table 9.1.2 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

9.7.7 Quality of Life Assessment

A patient-reported outcome (PRO) questionnaire will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the patient signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	**Haematology**	**Urine Tests**	**Other**
Albumin	Haemoglobin	Appearance	CRP
Alkaline phosphatase	Haematocrit	Color	
ALT (SGPT)	WBC count	pH	**Coagulation Screen including:**
AST (SGOT)	RBC count	Specific gravity	APTT
Direct bilirubin	Platelet count	Ketones	PT/INR
Total bilirubin	Differential cell count	Protein	TT
BUN		Glucose	
Calcium		Bilirubin	
Chloride		Nitrite	
Total cholesterol		Urobilinogen	
CO_2		Haemoglobin	
Creatinine			
Glucose			
GGT			
LDH			
Phosphorus			
Potassium			
Total protein			
Sodium			
Uric acid			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

9.7.8.3 Liver function and Hepatitis Testing

Patients will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. If medical records showing no evidence of Hepatitis B or Hepatitis C infection from the previous 30 days are available, then the Screening tests do not need to be done.

Evidence of ongoing infection (positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) will be an exclusion criteria. If these parameters are negative and therefore no active infection is detected, then the patients may be included if they were vaccinated (for hepatitis B) or cleared the infection (for hepatitis C) and their liver function is acceptable.

A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- Alkaline phosphatase above the normal cut off.
- INR over 1.4.
- Thrombocytopoenia under 100 x 10^9/L

9.7.8.4 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored hourly for 6 hours and then every 2 hours for 6 hours and then at 4 hour intervals.

A complete physical examination is necessary during Screening/Baseline; thereafter, brief physical examinations may be performed at the discretion of the investigator based on the patient's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems (level of consciousness, speech, language, cranial nerves, motor strength, motor tone, abnormal movements, reflexes, upper extremity sensation, lower extremity sensations, gait, Romberg, nystagmus, and coordination).

A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, and gastrointestinal assessments.

Weight and height will be recorded at Screening only.

9.7.8.5 Viral Shedding

Viral shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Eng.J.Med (Supplemental Appendix); Manno, 2006, Nature Med. (Supplemental Appendix); Schenk-Braat, 2007, J Gene Med; Croteau, 2004, Ann Occup Hyg). It has been constantly shown that the vector was not detectable anymore at 40 days, with its persistence in semen for 16 weeks in the seminal fluid but not in motile sperm (Manno, 2006, Nature Med.). In addition, it has always involved only an extremely small fraction of the injected dose.

Viral shedding will also be extensively studied in the present clinical trial, every week up to 16 weeks, then every 4 weeks to one year, then every three months. During the Post-Infusion Follow-Up period, patients will undergo testing of various bodily fluids to look for evidence of viral shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2 and Table 9.1.3. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a patient administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the patient that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the patient is a haemophiliac) should not be recorded as AEs but will instead be captured in patient diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening

Note: Life-threatening refers to an event that places the patient at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.

- Requires or prolongs inpatient hospitalization. Hospitalization for less than 24 hours will not be considered to be an SAE. Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a patient exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the patient or require intervention to prevent one of the above consequences (e.g. anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of liver enzymes triggering a corticosteroid treatment

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs and SAEs and EOSI, at each patient contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the patient's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the patient or qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments should be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.2. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred OR • The administration of the IP and the occurrence of the AE are not reasonably related in time OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time AND • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time AND • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation time points. Such an event should be recorded only once on the AE eCRF unless its severity increases or decreases (in which case it should be recorded again on the AE eCRF).

A recurrent AE is one that occurs and resolves between patient evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as AE if **any** of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The laboratory abnormality persists upon repeat confirmatory testing.
- The abnormality suggests a disease and/or organ toxicity

- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a patient taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in partner should be reported within 24 hours of the site becoming aware of the pregnancy by faxing the Pregnancy Form in the study reference materials to BPV.
The Investigator must make every effort to follow the patient through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study patient, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BioMarin Pharmacovigilance (BPV)

within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

The reporting period for SAEs begins after informed consent is obtained and the first administration of study drug is given. It continues for approximately 5 years or until study discontinuation/termination, whichever is longer.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Patients after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the patient is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the patient's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each patient to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the patient's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a patient has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a patient that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect patients against any immediate hazards that may affect the safety of patients, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax: PI
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

The chromogenic FXa assay and the APTT assays are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place a within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, Hepatitis B, Hepatitis C, and HIV. Patients with documented negative results within the last 30 days do not need to be retested.
- Complete Physical Exam (including height and weight)
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic FXa (plasma)
    - Baseline hFVIII activity level – one-stage APTT assay
    - hFVIII coagulation activity exploratory assay
    - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
    - hFVIII total antibody assay
    - hFVIII antigen (ELISA)
- Blood sample for AAV5 Assays

- AAV5 antibody titer
- AAV5 transduction inhibition assays

- Screen for Hepatitis B, Hepatitis C, and HIV if required (Patients with documented negative results within the last 30 days do not need to be retested)
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Blood samples for Biomarker testing (HLA typing, FVIII mutation status, TNFα and IL10a single nucleotide polymorphisms)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)
- Samples for FVIII Assays
  - FVIII activity – chromogenic FXa (plasma)
  - FVIII activity level –APTT One stage assay
  - FVIII coagulation activity exploratory assay
  - FVIII inhibitor level (Bethesda assay with Nijmegen modification)
  - FVIII antibody assay
  - FVIII antigen (ELISA)
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Mononuclear cell collection for CTL baseline
- PCR of vector genomes in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each patient. Patients will be hospitalized for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assays
- BMN 270 Infusion
- Vital Signs
    - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the patient's hospitalization.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, patients will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Every Visit

Every week (Weeks 1 through 16), the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
    - Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

- Samples for FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- FVIII antibody titer
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Collection to occur on Day 1, 3, 7 following BMN 270 infusion, and then weekly until 3 consecutive negative results are obtained
- Exploratory biomarker assessments

12.5.2 Every 4 Weeks

Every 4 weeks (Weeks 4, 8, 12, and 16), the following procedure will be performed:

- Mononuclear cell collection

12.5.3 Weeks 1, 3, and 6

At Weeks 1, 3, and 6, the following procedure will be performed:

- Test for Hepatitis B and Hepatitis C reactivation

12.5.4 Weeks 1, 8, and 16

At Weeks 1, 8, and 16, the following procedure will be performed:

- AAV5 antibody titer

12.5.5 Weeks 1, 2, 3, 4, and 16

At Weeks 1, 2, 3, 4, and 16, the following procedure will be performed:

- QoL assessment

12.6 Safety Follow-Up – Year 1

After the 16 weekly infusion follow-up visits are complete, subjects will return to the study site at Weeks 20, 28, 36, 44, and 52 (± 48 hours), when the following procedures will be completed:

12.6.1 Every Visit

At Weeks 20, 28, 36, 44, and 52, the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.2 Weeks 28 and 52

At Weeks 28 and 52, the following procedure will be performed:

- QoL assessment

12.7 Safety Follow-Up – Years 2-5

During Years 2-5 of Safety Follow-up, patients will be assessed every 3 months (± 72 hours). At these times, the following procedures will be completed:

12.7.1 Every Visit

Every 3 months (± 72 hours), the following procedures will be performed:

- Physical exam

- Complete Physical Exam will be performed every 52 weeks; Brief Physical Exams may be performed at other visits.

- Assessment of Adverse Events and Concomitant Medications
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
  - FVIII activity level (APTT)
  - FVIII activity level (FXa chromogenic assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
  - FVIII activity confirmation (Frozen plasma)
  - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
  - Sample testing during Safety Follow-Up is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs

12.7.2 Every Other Visit (Every 6 Months)

Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedure will be performed:

- QOL assessment

12.8 Early Termination Visit

The Early Termination visit will occur on the date the patient withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a patient leaves the study prior to the Week 270 visit, the patient will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications

- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs
- QOL assessment

12.9 End of Study

The study will end after the last patient completes the last Safety Follow-Up visit (Week 270). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study patients may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all patients having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The patients will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of patients, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay between 1 to 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

14.4 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.5 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-patient listing will be provided for those patients who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of patients who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.6 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 12 patients may be dosed in the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

14.7 Analysis Populations

The Safety analysis population is defined as all enrolled patients who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled patients who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a patient's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on patient risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to patients, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active patients must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE

There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the investigator representatives and the Sponsor will be established.

The DRB will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess available patient safety and efficacy data and make recommendations with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of patient safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study patients to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study patient's disease that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study patient has been injured by the IP or by procedures performed as part of the study. Any patient who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The patient should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the patient's health insurance company or other third party payer for the cost of this medical treatment. If the patient has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each patient. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and patient records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Patients must also allow access to their medical records, and patients will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to patient records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a patient's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon

completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study patients, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, patient charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain patient identifiers for at least 15 years after the completion or discontinuation of the study. Patient files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of patient files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

21 REFERENCES

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB/IEC/REB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201, Amendment 2

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

[redacted]

Medic[redacted]

Printed name: [redacted] MD, PhD, [redacted] Clinical Sciences_____

PI [redacted]

PI [redacted]

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See pages 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Text Revisions	Rationale
2/Synopsis/(Diagnosis and All Criteria for Inclusion and Exclusion)	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 2. Any evidence of ~~detectable viral load of~~ active infection or any immunosuppressive disorder.	1
2/Synopsis/(Diagnosis and All Criteria for Inclusion and Exclusion)	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 3. Patients that are HIV positive are excluded.	1
9.1/Overall Study Design and Plan	Additionally, if any of the events listed in Section 9.3.3.1 occur, enrollment into the trial will be halted to complete an extensive safety analysis. Notification of the study halt will be sent by the Sponsor to the participating sites via telephone and email immediately after becoming aware of a safety concern. This will ensure no additional subjects are dosed until the signal has been completely evaluated. If, following safety review by the DRB and the Sponsor, it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data will be submitted to the health authority.	2
9/Investigational Plan/(Table 9.1.1 – Schedule of Events – Screening and Infusion)	~~FVIII Treatment Washout[b]~~	6
9/Investigational Plan/(Table 9.1.1 – Schedule of Events – Screening and Infusion)/Footnotes	~~[b] Patients will have a washout period of 72 hours prior to BMN 270 infusion. If a bleeding occurs, another washout period of 72 hours will be performed. If another bleeding occurs during this second washout period, then the patient may be excluded from the study at the physician's discretion.~~	6
9/Investigational Plan/(Table 9.1.1 – Schedule of Events – Screening and	~~[b]~~Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA). ~~hFVIII assays should be assessed only when the patient has been off FVIII therapy for at least the previous 4 days.~~	6

Section No./Title	Text Revisions	Rationale
Infusion)/Footnotes		
9.3.2/Exclusion Criteria	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 2. Any evidence of ~~detectable viral load of~~ active infection or any immunosuppressive disorder.	1
9.3.2/Exclusion Criteria	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 3. Patients that are HIV positive are excluded.	1
9.3.3.1/Study Safety Evaluation Criteria	If any of the following events occur (except the persistence of AAV5) in a patient in the study who has received BMN 270 infusion, enrollment into the trial will be put on halt to complete an extensive safety analysis ~~will be performed and BioMarin and the Principal Investigator will discuss pursuing the trial or increasing the time before the next patient is enrolled.~~ per Section 9.1.	2
9.4.8.1/Concomitant Haemophilia Treatments	**Concomitant Haemophilia Treatments ~~are discussed in the washout section below (Section).~~** ~~**9.4.8.2 Factor VIII Washout**~~ ~~Patients will have a washout period of 72 hours prior to the BMN 270 infusion. If a bleeding occurs, another washout period of 72 hours will be performed. If another bleeding occurs during this second washout period, then the patient may be excluded from the study at the physician's discretion.~~ Patients on "on demand" therapy for FVIII will continue their treatment at will. Patients on prophylactic FVIII therapy will discontinue their treatment starting the day of infusion and switch to an "on demand" schedule. FVIII can always be taken as needed by the patient, who will carefully record his treatment and bleeding episodes in his diary.	6
9.7.2/Primary Efficacy Variables	To measure the primary efficacy variable, the following assays will be used: • FVIII activity (chromogenic FXa assay) • FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time) • ~~Exploratory FVIII activity assay~~	4
9.7.8.2/Clinical Laboratory Assessments	All abnormal clinical laboratory ~~result pages~~ results should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant.	Admin.

Section No./Title	Text Revisions	Rationale
10.1.1/Adverse Events	• Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures~~, medication washout, or no-treatment run-in~~).	6
10.5.1/Expedited Reporting Requirements	The Sponsor is responsible for identifying, preparing and reporting all SUSARs to the relevant competent authorities, ethics committees and investigators in accordance with the requirements identified in the Clinical Trials Regulations.	3
11/Appropriateness of Measurements	The chromogenic FXa assay and the APTT assays are both validated and utilize CE marked reagents. The exploratory FVIII activity assay will be used for exploratory purposes only.	4
12.2/Screening Visit	• Samples for hFVIII Assays o Baseline hFVIII activity – chromogenic FXa (plasma) ~~(at least 72 hours off therapy)~~ o Baseline hFVIII activity level – one-stage APTT assay ~~(at least 72 hours off therapy)~~	6
12.2/Screening Visit	• Screen for Hepatitis B, Hepatitis C, and HIV if required (Patients with documented negative results within the last 30 days do not need to be retested)	Admin.
12.3/Baseline Visit	~~• FVIII Treatment Washout (refer to Section)~~	6
12.3/Baseline Visit	• Samples for FVIII Assays o FVIII activity – chromogenic FXa (plasma) ~~(at least 72 hours off therapy)~~ o FVIII activity level –APTT One stage assay ~~(at least 72 hours off therapy)~~	6
15/Data Monitoring Committee	There will be no formal DMC for this study, however a safety and efficacy evaluation board (the Data Review Board [DRB]) composed of the ~~investigators~~ investigator representatives and the Sponsor will be established. The ~~primary responsibility of the board will be~~ Data Review Board [DRB] will review safety and efficacy on an ongoing basis. The DRB will meet prior to dose escalation or dose expansion to assess ~~patient's~~ available patient safety and efficacy data ~~accumulated for a particular dose level and to~~ and make recommendations ~~regarding dose escalation.~~ with regards to the conduct of study. Should a safety signal arise, including one which may warrant a halt of study enrollment, the DRB will promptly convene for further assessment of patient safety. Notification of all DRB meetings and meeting outcomes will be sent to participating sites.	5

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Patient Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015
Date of Amendment 1:	06 March 2015

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 1

Date: 06 March 2015

RATIONALE AND SUMMARY OF MAJOR CHANGES

A summary of major changes covered by Amendment 1 to the BMN 270-201 protocol is provided below.

1. Addition of "Receipt of any vector or gene transfer agent" to exclusion criteria.

Rationale: MHRA scientific advice in May 2014 stated that "a rationale has to be provided as to why receipt of any vector or gene transfer agent at any time is not an absolute exclusion criterion, rather than limiting to any vector/gene transfer agent that had been received in the previous 6 months."

2. Drug substance "AAV5-SQ-codop-hFVIII" has been revised to "AAV5-hFVIII-SQ."

Rationale: This change was made to remain consistent with drug substance description in the IMPD and the IB.

3. Appendix 1 has been removed.

Rationale: Appendix 1 contained a detailed schedule of assessments that is not the schedule for this study. Instead, a reference has been made to the Nathwani 2014 supplemental information available online.

4. Appendix 2 has been removed.

Rationale: Appendix 2 contained unpublished data. Instead, a reference has been added to Manno, 2006 supplemental information which includes vector biodistribution data by tissue and vector dose.

5. Inclusion Criterion concerning severe level of FVIII deficiency has been modified to state that "Patients will be considered as severe if their FVIII baseline level is 1 IU/dL or less"

Rationale: Changed description to more accurately describe patients with severe Haemophilia A. Baseline level is never "reached"; it is historical and based on their mutation.

6. Minor edits for clarity and consistency have been incorporated.

Specific changes included in this amendment, including the Synopsis, since the original protocol (approved 10 February 2015) are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for rhFVIII (under 24 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis up to 30 to 50 for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death. Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and

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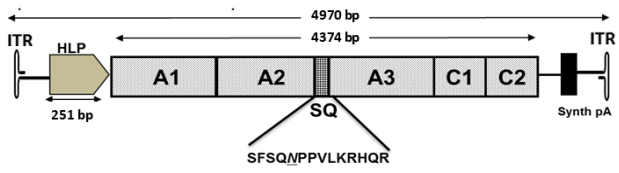
thus, show promise in reduced dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 1).

Figure 1. BMN 270 transgene construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue.

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The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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 expression of 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

STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled sequentially into one of up to three cohorts according to dose level:

1. 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
2. 2E13 vg per kilogram, iv

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3. 6E13 vg per kilogram, iv

Patients will be enrolled sequentially every 3 weeks or more between cohorts. Dose escalation may occur after a single patient has been safely dosed if the resulting FVIII activity at Week 3 is < 5 IU/dL. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the patient numbers exposed to subtherapeutic doses.

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity is ≥ 5 IU/dL, then the other patients of the dose group will be enrolled without waiting for 3 weeks between patients.

Patient 1 will be dosed by intravenous perfusion with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥ 5 IU/dL at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥ 5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Because patients develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a treatment option and these patients have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dose. Any safety signal will trigger a review of the data and possible additional analysis including mononuclear collection.

NUMBER OF PATIENTS PLANNED:

Up to 12 patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

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DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII baseline level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitates for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, or no inhibitor on 2 consecutive occasions within the past 12 months using a modified Nijmegen Bethesda assay higher than 0.6 Bethesda Unit (BU)
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition and AAV5 total antibodies
2. Evidence of detectable viral load of HIV
3. Significant liver dysfunction as defined by abnormal elevation of ALT (alanine transaminase) to 3 times the upper limit of normal, bilirubin, alkaline phosphatase, or an INR (international normalized ratio) of 1.4. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
4. Evidence of any bleeding disorder not related to Haemophilia A
5. Platelet count of $< 100 \times 10^9$/L
6. Creatinine $\geq$ 1.5 mg/dL
7. Liver cirrhosis of any etiology as assessed by liver ultrasound
8. Hepatitis B if surface antigen is positive
9. Hepatitis C if RNA is positive

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10. Treatment with any IP within 30 days prior to the screening visit
11. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
12. Receipt of any vector or gene transfer agent

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each patient will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous perfusion.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver function tests (LFT)

No major toxicity is expected based on preclinical studies in mice and monkeys. An asymptomatic transaminitis was observed at week 8-10 after administration with an AAV8-FIX, providing the rationale for the following surveillance plan. Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.

There will be a detailed monitoring plan for assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Patients will be asked to keep a patient diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be

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used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 12 patients may be dosed in the study. The patients will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at $\geq$ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay between 1 to 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as viral shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
eCRF	electronic case report form
DMC	Data Monitoring Committee
ED	exposure days
EOSI	events of special interest
ETV	early termination visit
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
HA	Haemophilia A
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee

IND	Investigational New Drug (application)
INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LFT	liver funtion test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
vg	vector genomes

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the patients, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all patient recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible patients for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the patients will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each patient will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Patient Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each patient prior to entering the patient into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF , and if applicable, a copy of the approved patient information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each patient and will maintain the original in the record file of the patient.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to patients. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of patients in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each patient as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Laboratory evaluations will be performed at the local laboratories associated with the study sites. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to around 20 hours (Pipe, 2010, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration or problems of FVIII inhibitor formation, which occurs in 30% of patients on standard FVIII replacement therapy (Nathwani, 1992, Baillieres Clin.Haematol.).

Gene therapy offers the potential of a cure through continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2012, Haemophilia); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Paneda, 2009, Hum.Gene Ther.). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents > 30-fold safety factor from the no observed adverse effect level (NOAEL) in enabling nonclinical studies.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Eng.J.Med.; Nathwani, 2014, N.Eng.J.Med.). The large size of the FVIII cDNA was shortened and a

preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is also used in clinical trials and has shown good safety in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola. 2014, J Hepatology).

AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin. Haematol). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. . The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for rhFVIII (under 24 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis up to 30 to 50 for on demand treatment) (Nagel, 2011, Haemophilia). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need

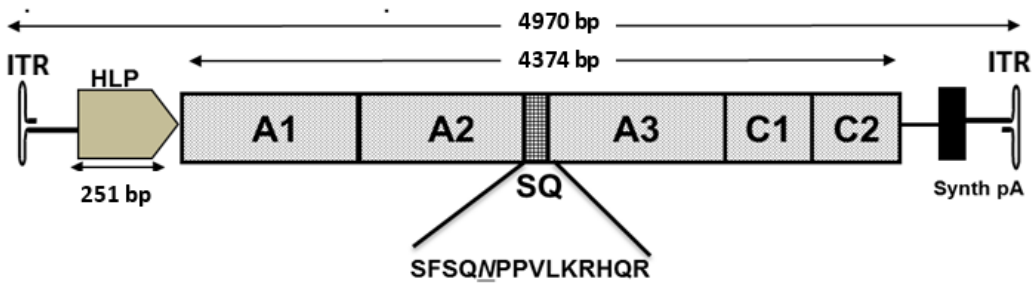
for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Pipe, 2010, Blood). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector(Nathwani, 2011, NEJM). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.). (Bainbridge, 2008, NEJM; Maguire, 2009, Lancet; Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: BMN 270 Transgene Construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 8-10 after administration with an AAV8-FIX, providing the rationale for the following surveillance plan (Manno, 2006, Nature Med.). Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that potentially could contribute to a better opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey. A detectable pharmacological response based on activity was observed at 6E12 vg/kg.

A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to patients but may potentially benefit the patient therapeutically.

8 STUDY OBJECTIVES

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 expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled sequentially into one of up to three cohorts according to dose level:

1. 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
2. 2E13 vg per kilogram, iv
3. 6E13 vg per kilogram, iv

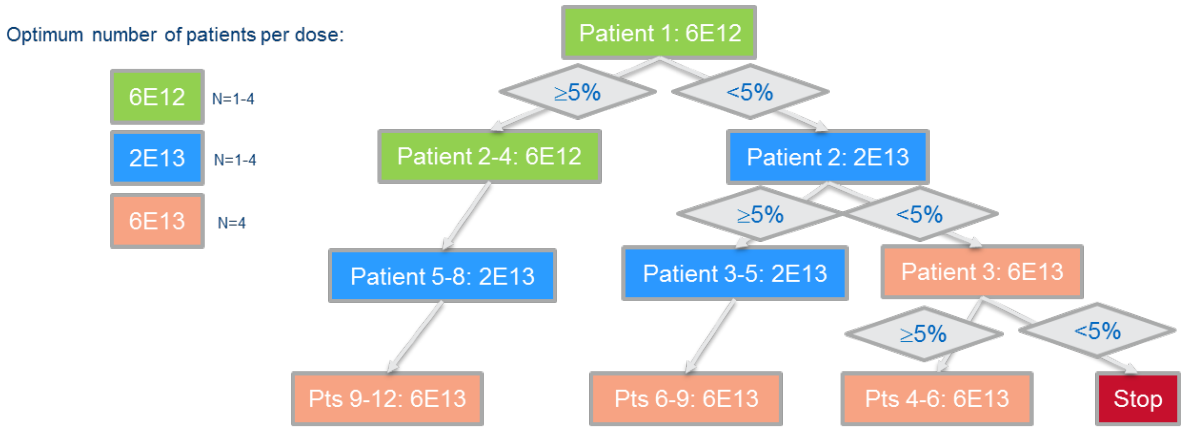
Patients will be enrolled sequentially every 3 weeks or more between cohorts. Dose escalation may occur after a single patient has been safely dosed if the resulting FVIII activity at Week 3 is < 5 IU/dL. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the patient numbers exposed to subtherapeutic doses.

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity is ≥5 IU/dL, then the other patients of the dose group will be enrolled without waiting for 3 weeks between patients.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme



Patient 1 will be dosed by intravenous perfusion with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥5 IU/dL (expressed as % in Figure 9.1.1) at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and if no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Because patients develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a treatment option and these patients have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dose. Any safety signal will trigger a review of the data and possible additional analysis including mononuclear collection.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 for Safety Follow-up.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion		BMN 270 Infusion Visit (Day 1)
	Screening (Day -28 to Day -1)	**Baseline (Day -7 to Day -1)[i]**	
Informed consent	X		
Medical History	X		
Physical Exam[a]	X	X	X
Vital Signs	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X
Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)	X	X	
Electrocardiogram	X		
Chest X-ray	X		
Liver Ultrasound	X		
FVIII Treatment Washout[b]		X	
hFVIII Assays[c]	X	X	
AAV5 Assays[d]	X		X
Screen for Hepatitis B, Hepatitis C, HIV[e]	X		
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[f]	X	X	
Mononuclear cell collection for CTL baseline		X	
PCR of vector genomes in blood, saliva, urine, semen, and stools		X	
Biomarker testing[g]	X		
Exploratory biomarker assessments[h]		X	
Quality of Life (QoL) assessment		X	
BMN 270 Infusion			X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height and weight will be recorded at Screening only.

[b] Patients will have a washout period of 72 hours prior to BMN 270 infusion. If a bleeding occurs, another washout period of 72 hours will be performed. If another bleeding occurs during this second washout period, then the patient may be excluded from the study at the physician's discretion.

[c] Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA). hFVIII assays should be assessed only when the patient has been off FVIII therapy for at least the previous 4 days.

[d] Screening (done during Screening) and testing (at Infusion Visit) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays). The assessment on the day of the infusion visit must be performed before the BMN 270 infusion is given.

[e] Patients with documented negative results within the last 30 days do not need to be retested.

[f] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[g] Includes HLA typing, FVIII mutation status, TNFα and IL10a single nucleotide polymorphisms.

[h] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

[i] Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up

	Follow-Up After BMN 270 Administration - Weeks*															
Assessment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical exam[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
hFVIII activity assays[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
hFVIII antibody titer	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Exploratory biomarker assessments[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
QoL assessment	X	X	X	X												X
AAV5 antibody titer	X							X								X
Testing for reactivation of hepatitis B and hepatitis C	X		X			X										
Mononuclear cell collection				X				X				X				X

* Visit windows are ±48 hours

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included. Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

[c] Includes hFVIII activity level (APTT and FXa chromogenic assay), hFVIII activity confirmation (frozen plasma), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA).

[d] Collection to occur on Day 1, 3, 7 following BMN 270 infusion, and then weekly until 3 consecutive negative results are obtained

[e] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

Table 9.1.3: Schedule of Events – Safety Follow-Up

Assessment	Year 1 - Weeks*					Years 2-5*	ETV (early termination visit)
	20	28	36	44	52	Q3M	
Physical exam[a]	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]	X	X	X	X	X	X	X
hFVIII activity assays[c]	X	X	X	X	X	X	X
AAV5 antibody titer	X	X	X	X	X	X	X
hFVIII antibody titer	X	X	X	X	X	X	X
Mononuclear Cell Collection (for determination of FVIII and Capsid specific CTL activity)	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X
QoL assessment		X			X	X[e]	X

* Visit windows are ±72 hours

[a] Complete physical examination should be performed at Weeks 52 and every 52 weeks thereafter; brief physical exam may be performed at other study visits. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), hFVIII activity confirmation (frozen plasma), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA).

[d] Sample testing during Safety Follow-Up is not required if 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[e] QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Three doses of BMN 270 will be evaluated and the dose escalation decision tree is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

There will be no control group. Parameters for each patient will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 12 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII baseline level is 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitates for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, or no inhibitor on 2 consecutive occasions within the past 12 months using a modified Nijmegen Bethesda assay higher than 0.6 Bethesda Unit (BU)

6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition and AAV5 total antibodies
2. Evidence of detectable viral load of HIV
3. Significant liver dysfunction as defined by abnormal elevation of ALT (alanine transaminase) to 3 times the upper limit of normal, bilirubin, alkaline phosphatase, or an INR (international normalized ratio) of 1.4. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
4. Evidence of any bleeding disorder not related to Haemophilia A
5. Platelet count of $< 100 \times 10^9$/L
6. Creatinine ≥ 1.5 mg/dL
7. Liver cirrhosis of any etiology as assessed by liver ultrasound
8. Hepatitis B if surface antigen is positive
9. Hepatitis C if RNA is positive
10. Treatment with any IP within 30 days prior to the screening visit
11. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.
12. Receipt of any vector or gene transfer agent

9.3.3 Removal of Patients from Treatment or Assessment

Patients may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any patient who requests to be withdrawn. A patient's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all patient withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative

reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a patient from the study include, but are not limited to, the following:

- Patient requires medication or medical procedure prohibited by the protocol
- Patient does not adhere to study requirements specified in the protocol
- Patient was erroneously admitted into the study or does not meet entry criteria
- Patient is lost to follow-up

If a patient fails to return for scheduled visits, a documented effort must be made to determine the reason. If the patient cannot be reached by telephone, a certified letter should be sent to the patient requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each patient, before enrollment into the study, that for evaluation of study results, the patient's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each patient. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the patient and the patient will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur (except the persistence of AAV5) in a patient in the study who has received BMN 270 infusion, an extensive safety analysis will be performed and BioMarin and the Principal Investigator will discuss pursuing the trial or increasing the time before the next patient is enrolled.

- A 5-fold increase in ALT after BMN 270 administration
- The occurrence of other drug-related Grade III-IV toxicity, including liver failure and clinical hepatitis
- Drug-related Grade II toxicity that persists for at least 7 days
- The occurrence of neutralizing antibodies to hFVIII following BMN 270 infusion

- The persistent detection of the AAV vector genome in the semen of a participant more than 26 weeks after BMN 270 infusion, as discussed in Section 9.7.8.5
- The occurrence of a malignancy at any point after BMN 270 infusion that is assessed as possibly, probably, or definitely related to the study agent
- Any unexplained serious adverse event ≥ grade 4 assessed as at least possibly related to study drug

9.3.4 Patient Identification and Replacement of Patients

Each patient will be assigned a unique patient identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Patients who withdraw from the study after Week 16 will not be replaced.

9.3.5 Duration of Patient Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be

stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical exam performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (e.g. the median cubital vein) and flushed with saline. FVIII protein concentrate will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Patients will remain hospitalized for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours of hospitalization, participants will be discharged unless toxicity has been observed in which case hospitalization may be extended or transfer to an outpatient facility may occur based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Patients to Treatment Groups

Patients who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study patient. Upon their enrollment into the study, patients will be assigned a unique patient number and dose level by the Sponsor.

Patients are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Patients may receive the next higher dose if the previous patient does not meet the activity criteria, or the same dose if the previous

patient meets the activity criteria. Up to 6 patients can have the same dose, for a total of 12 patients in this study.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in non-human primates. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Patient

A minimum of three weeks are required between patients, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next patient will be made as described below.

9.4.6.2 Selection of Dose for Each Patient

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

Patient 1 will be dosed with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥5 IU/dL at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and if no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Refer to Figure 9.1.1 for a visual representation of the study design.

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications taken by a patient for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Immunosuppressive agents

Haemophilia treatments are discussed in the washout section below (Section 9.4.8.1).

9.4.8.1 Factor VIII Washout

Patients will have a washout period of 72 hours prior to the BMN 270 infusion. If a bleeding occurs, another washout period of 72 hours will be performed. If another bleeding occurs during this second washout period, then the patient may be excluded from the study at the physician's discretion.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period, each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

Reports of abnormal LFTs (defined as 1.5x the patient's baseline level) should be made to BioMarin within 24 hours of the lab value being available. If the LFTs are abnormal, treatment with prednisolone initiated at a dose of 60 mg per day and then gradually tapered (60 mg daily for the first week, then 40 mg the second week, then 30 mg the third week, then 20 mg the fourth week, then stop) will be started immediately.

9.4.9 Treatment Compliance

Study drug will be administered to patients at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received, patients to whom IP is dispensed (patient-by-patient dose specific accounting), and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to stable expression of FVIII at 5 IU/dL, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes (Nathwani, 1992, Baillieres Clin.Haematol.).

To measure the primary efficacy variable, the following assays will be used:

- FVIII activity (chromogenic FXa assay)
- FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time)
- Exploratory FVIII activity assay

The FVIII activity level and the number of patients with FVIII activity ≥ 5 IU/dL will be summarized.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Patients will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the patient's diary or other patient records.

Assessment of viral shedding at one week after BMN 270 infusion will also be considered an efficacy variable.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 6-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to an immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage APTT and/or chromogenic FXa assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2 and Table 9.1.3.

9.7.6 Exploratory Biomarker Assessments

Blood samples will be collected from subjects at the time points indicated in Table 9.1.1 and Table 9.1.2 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

9.7.7 Quality of Life Assessment

A patient-reported outcome (PRO) questionnaire will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the patient signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	**Haematology**	**Urine Tests**	**Other**
Albumin	Haemoglobin	Appearance	CRP
Alkaline phosphatase	Haematocrit	Color	
ALT (SGPT)	WBC count	pH	**Coagulation Screen including:**
AST (SGOT)	RBC count	Specific gravity	APTT
Direct bilirubin	Platelet count	Ketones	PT/INR
Total bilirubin	Differential cell count	Protein	TT
BUN		Glucose	
Calcium		Bilirubin	
Chloride		Nitrite	
Total cholesterol		Urobilinogen	
CO_2		Haemoglobin	
Creatinine			
Glucose			
GGT			
LDH			
Phosphorus			
Potassium			
Total protein			
Sodium			
Uric acid			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

9.7.8.3 Liver function and Hepatitis Testing

Patients will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. If medical records showing no evidence of Hepatitis B or Hepatitis C infection from the previous 30 days are available, then the Screening tests do not need to be done.

Evidence of ongoing infection (positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) will be an exclusion criteria. If these parameters are negative and therefore no active infection is detected, then the patients may be included if they were vaccinated (for hepatitis B) or cleared the infection (for hepatitis C) and their liver function is acceptable.

A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- Alkaline phosphatase above the normal cut off.
- INR over 1.4.
- Thrombocytopoenia under 100 x 10^9/L

9.7.8.4 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored hourly for 6 hours and then every 2 hours for 6 hours and then at 4 hour intervals.

A complete physical examination is necessary during Screening/Baseline; thereafter, brief physical examinations may be performed at the discretion of the investigator based on the patient's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems (level of consciousness, speech, language, cranial nerves, motor strength, motor tone, abnormal movements, reflexes, upper extremity sensation, lower extremity sensations, gait, Romberg, nystagmus, and coordination).

A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, and gastrointestinal assessments.

Weight and height will be recorded at Screening only.

9.7.8.5 Viral Shedding

Viral shedding has been extensively studied in all similar clinical trials performed to date. (Nathwani, 2014, N.Eng.J.Med (Supplemental Appendix); Manno, 2006, Nature Med. (Supplemental Appendix); Schenk-Braat, 2007, J Gene Med; Croteau, 2004, Ann Occup Hyg). It has been constantly shown that the vector was not detectable anymore at 40 days, with its persistence in semen for 16 weeks in the seminal fluid but not in motile sperm (Manno, 2006, Nature Med.). In addition, it has always involved only an extremely small fraction of the injected dose.

Viral shedding will also be extensively studied in the present clinical trial, every week up to 16 weeks, then every 4 weeks to one year, then every three months. During the Post-Infusion Follow-Up period, patients will undergo testing of various bodily fluids to look for evidence of viral shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2 and Table 9.1.3. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a patient administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the patient that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the patient is a haemophiliac) should not be recorded as AEs but will instead be captured in patient diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening

  Note: Life-threatening refers to an event that places the patient at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization. Hospitalization for less than 24 hours will not be considered to be an SAE. Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a patient exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the patient or require intervention to prevent one of the above consequences (e.g. anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of liver enzymes triggering a corticosteroid treatment

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs and SAEs and EOSI, at each patient contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the patient's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the patient or qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments should be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.2. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred OR • The administration of the IP and the occurrence of the AE are not reasonably related in time OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time AND • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time AND • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation time points. Such an event should be recorded only once on the AE eCRF unless its severity increases or decreases (in which case it should be recorded again on the AE eCRF).

A recurrent AE is one that occurs and resolves between patient evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the AE eCRF.

A clinical laboratory abnormality is considered clinically significant and should be documented as AE if **any** of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The laboratory abnormality persists upon repeat confirmatory testing.
- The abnormality suggests a disease and/or organ toxicity

- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a patient taking trial medication should be reported expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in partner should be reported within 24 hours of the site becoming aware of the pregnancy by faxing the Pregnancy Form in the study reference materials to BPV.
The Investigator must make every effort to follow the patient through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study patient, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BioMarin Pharmacovigilance (BPV)

within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

The reporting period for SAEs begins after informed consent is obtained and the first administration of study drug is given. It continues for approximately 5 years or until study discontinuation/termination, whichever is longer.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Patients after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the patient is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the patient's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each patient to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the patient's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a patient has discontinued or terminated study participation, if the Investigator believes that the death or SAE may have been related to prior study treatment. The Sponsor should

also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a patient that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect patients against any immediate hazards that may affect the safety of patients, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.9 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax: PI
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place a within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, Hepatitis B, Hepatitis C, and HIV. Patients with documented negative results within the last 30 days do not need to be retested.
- Complete Physical Exam (including height and weight)
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic FXa (plasma) (at least 72 hours off therapy)
    - Baseline hFVIII activity level – one-stage APTT assay (at least 72 hours off therapy)
    - hFVIII coagulation activity exploratory assay
    - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)
    - hFVIII total antibody assay

- hFVIII antigen (ELISA)

- Blood sample for AAV5 Assays
  - AAV5 antibody titer
  - AAV5 transduction inhibition assays
- Screen for Hepatitis B, Hepatitis C, and HIV if required
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Blood samples for Biomarker testing (HLA typing, FVIII mutation status, TNFα and IL10a single nucleotide polymorphisms)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)
- FVIII Treatment Washout (refer to Section 9.4.8.1)
- Samples for FVIII Assays
  - FVIII activity – chromogenic FXa (plasma) (at least 72 hours off therapy)
  - FVIII activity level –APTT One stage assay (at least 72 hours off therapy)
  - FVIII coagulation activity exploratory assay
  - FVIII inhibitor level (Bethesda assay with Nijmegen modification)
  - FVIII antibody assay
  - FVIII antigen (ELISA)
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Mononuclear cell collection for CTL baseline
- PCR of vector genomes in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each patient. Patients will be hospitalized for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
    - AAV5 antibody titer
    - AAV5 transduction inhibition assays
- BMN 270 Infusion
- Vital Signs
    - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the patient's hospitalization.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, patients will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Every Visit

Every week (Weeks 1 through 16), the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
    - Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

- Samples for FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- FVIII antibody titer
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Collection to occur on Day 1, 3, 7 following BMN 270 infusion, and then weekly until 3 consecutive negative results are obtained
- Exploratory biomarker assessments

12.5.2 Every 4 Weeks

Every 4 weeks (Weeks 4, 8, 12, and 16), the following procedure will be performed:

- Mononuclear cell collection

12.5.3 Weeks 1, 3, and 6

At Weeks 1, 3, and 6, the following procedure will be performed:

- Test for Hepatitis B and Hepatitis C reactivation

12.5.4 Weeks 1, 8, and 16

At Weeks 1, 8, and 16, the following procedure will be performed:

- AAV5 antibody titer

12.5.5 Weeks 1, 2, 3, 4, and 16

At Weeks 1, 2, 3, 4, and 16, the following procedure will be performed:

- QoL assessment

12.6 Safety Follow-Up – Year 1

After the 16 weekly infusion follow-up visits are complete, subjects will return to the study site at Weeks 20, 28, 36, 44, and 52 (± 48 hours), when the following procedures will be completed:

12.6.1 Every Visit

At Weeks 20, 28, 36, 44, and 52, the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.2 Weeks 28 and 52

At Weeks 28 and 52, the following procedure will be performed:

- QoL assessment

12.7 Safety Follow-Up – Years 2-5

During Years 2-5 of Safety Follow-up, patients will be assessed every 3 months (± 72 hours). At these times, the following procedures will be completed:

12.7.1 Every Visit

Every 3 months (± 72 hours), the following procedures will be performed:

- Physical exam

  - Complete Physical Exam will be performed every 52 weeks; Brief Physical Exams may be performed at other visits.

- Assessment of Adverse Events and Concomitant Medications
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
  - FVIII activity level (APTT)
  - FVIII activity level (FXa chromogenic assay)
  - FVIII coagulation activity exploratory assay
  - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
  - FVIII activity confirmation (Frozen plasma)
  - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
  - Sample testing during Safety Follow-Up is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs

12.7.2 Every Other Visit (Every 6 Months)

Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedure will be performed:

- QOL assessment

12.8 Early Termination Visit

The Early Termination visit will occur on the date the patient withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a patient leaves the study prior to the Week 270 visit, the patient will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications

- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs
- QOL assessment

12.9 End of Study

The study will end after the last patient completes the last Safety Follow-Up visit (Week 270). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study patients may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all patients having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The patients will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of patients, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at $\geq$ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay between 1 to 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 72 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

14.4 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.5 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-patient listing will be provided for those patients who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of patients who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.6 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 12 patients may be dosed in the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

14.7 Analysis Populations

The Safety analysis population is defined as all enrolled patients who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled patients who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a patient's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on patient risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to patients, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active patients must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE

There will be no DMC for this study, however a safety and efficacy evaluation board composed of the investigators and sponsor will be established. The primary responsibility of the board will be to assess patient's safety and efficacy data accumulated for a particular dose level and to make recommendations regarding dose escalation.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study patients to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study patient's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study patient has been injured by the IP or by procedures performed as part of the study. Any patient who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The patient should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the patient's health insurance company or other third party payer for the cost of this medical treatment. If the patient has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each patient. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and patient records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Patients must also allow access to their medical records, and patients will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to patient records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a patient's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to Locked. Upon

completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study patients, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, patient charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain patient identifiers for at least 15 years after the completion or discontinuation of the study. Patient files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of patient files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB/IEC/REB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

PI

Printed name: PI , MD, PhD, PI Clinical Sciences_____

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (See pages 2-3). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Text Revisions	Rationale
2/Synopsis (Objectives)	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-~~SQ codop~~ hFVIII-SQ) vector. • To determine the dose of AAV5-~~SQ codop~~ hFVIII-SQ required to achieve expression of 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 ~~rAAV5-SQ-codop~~AAV5-hFVIII-SQ	2
2/Synopsis (Diagnosis and All Criteria for Inclusion and Exclusion)	Individuals eligible to participate in this study must meet all of the following criteria: 1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII ~~levels have ever declined to~~baseline level is 1 IU/dL or less	5
2/Synopsis (Diagnosis and All Criteria for Inclusion and Exclusion)	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 12. Receipt of any vector or gene transfer agent	1
2/Synopsis (Statistical Methods)	We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of ~~96~~72 hours has elapsed since the last infusion of FVIII protein concentrates.	6
8/Study Objectives	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-~~SQ codop~~ hFVIII-SQ) vector. • To determine the dose of AAV5-~~SQ codop~~ hFVIII-SQ required to achieve expression of hFVIII at or above 5% of	2

Section No./Title	Text Revisions	Rationale
	normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product and AAV capsid proteins following systemic administration of ~~rAAV5-SQ-codop~~AAV5-hFVIII-SQ	
9.3.1/Inclusion Criteria	Individuals eligible to participate in this study must meet all of the following criteria: 1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII ~~levels have ever declined to~~baseline level is 1 IU/dL or less	5
9.3.2/Exclusion Criteria	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 12. Receipt of any vector or gene transfer agent	1
9.4.8.1/FVIII Washout	Patients will have a washout period of 72 hours prior to the BMN 270 infusion.	6
9.4.8.2/Glucocorticoid Treatment of Elevated Hepatic Transaminases	Reports of abnormal LFTs (defined as 1.5x the patient's baseline level) should be made to BioMarin within 24 hours of the lab value being available. If the LFTs are abnormal, treatment with prednisolone initiated at a dose of ~~60mg~~60 mg per day and then gradually tapered (60 mg daily for the first week, then 40 mg the second week, then 30 mg the third week, then 20 mg the fourth week, then stop) will be started immediately.	6
9.7.8.5/Viral Shedding	Viral shedding has been extensively studied in all similar clinical trials performed to date. ~~(; ;~~(Nathwani, 2014, N.Eng.J.Med (Supplemental Appendix); Manno, 2006, Nature Med.(Supplemental Appendix); Schenk-Braat, 2007, J Gene Med; Croteau, 2004, Ann Occup Hyg).	3/4/6
10.4.1.4/Abnormal Laboratory Values	A clinical laboratory abnormality is considered clinically significant and should be documented as AE if **any** of the following conditions is met: • The laboratory abnormality ~~is not otherwise refuted by a repeat test to confirm the abnormality~~persists upon repeat confirmatory testing.	6

Section No./Title	Text Revisions	Rationale
10.4.1.9/Pregnancy	Although not an AE per se, pregnancy in the partner of a patient taking trial medication should be reported ~~as an SAE~~expeditiously to the Sponsor to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion. Pregnancy in partner should be reported within 24 hours of the site becoming aware of the pregnancy by faxing the Pregnancy Form in the study reference materials to BPV. ~~In addition, pregnancy in a patient is also reported on the End of Study eCRF.~~	6

12.2/Screening Visit	• Samples for hFVIII Assays
	  ○ Baseline hFVIII activity – chromogenic FXa (plasma) (at least ~~96~~72 hours off therapy)
	  ○ Baseline hFVIII activity level –one-stage APTT assay (at least ~~96~~72 hours off therapy)
	• ~~FVIII Treatment Washout (refer to Section 9.4.8.1)~~
		6

12.3/Baseline Visit	• Samples for FVIII Assays
	  ○ FVIII activity – chromogenic FXa (plasma) (at least ~~96~~72 hours off therapy)
	  ○ FVIII activity level –APTT One stage assay (at least ~~96~~72 hours off therapy)
	• FVIII Treatment Washout (refer to Section 9.4.8.1)
		6

12.4/Treatment Visit	• Blood sample for AAV5 Assays (must be performed before the BMN 270 infusion)
	  ○ AAV5 antibody titer
	  ○ AAV5 transduction inhibition assays
		6

14.2/Primary and Secondary Efficacy Analysis	We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of ~~96~~72 hours has elapsed since the last infusion of FVIII protein concentrates.	6
21/References	Manno CS, Arruda VR, Pierce GF, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nature Med. 2006;12:342-347 and Supplemental Appendix, available at: http://www.nature.com/nm/journal/v12/n3/extref/nm1358-S3.pdf~~.~~.	3/6

Section No./Title	Text Revisions	Rationale
21/References	Nathwani AC, Reiss UM, Tuddenham EGD, et al. Long-Term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B. N.Engl.J.Med. 2014;371:1994-2004 and Supplemental Appendix, available at: http://www.nejm.org/doi/suppl/10.1056/NEJMoa1407309/suppl_file/nejmoa1407309_appendix.pdf.	4/6
Appendices	Removal of APPENDIX : NATHWANI APPENDIX 2014	3
Appendices	Removal of APPENDIX : AAV-SPECIFIC ISSUES RELATED TO VECTOR THERAPY (PI)	4

CLINICAL STUDY PROTOCOL

Study Title:	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
Protocol Number:	270-201
Active Investigational Product:	AAV5-hFVIII-SQ
IND/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number:	2014-003880-38
Indication:	Haemophilia A
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 1/2
Sponsor's Responsible Medical Monitor:	PI PI BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration of Patient Participation:	Approximately 264 weeks
Dose:	Varied
Study Population:	Males aged 18 or older
Date of Original Protocol:	10 February 2015

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

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

PROTOCOL NUMBER:

270-201

STUDY SITES:

Approximately 6-10 sites worldwide.

PHASE OF DEVELOPMENT:

Phase 1/2

STUDY RATIONALE:

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for rhFVIII (under 24 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis up to 30 to 50 for on demand treatment). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: BMN 270 NAME OF ACTIVE INGREDIENT: AAV5-hFVIII-SQ	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

death. Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough. However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise. They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector. Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life.

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 1).

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
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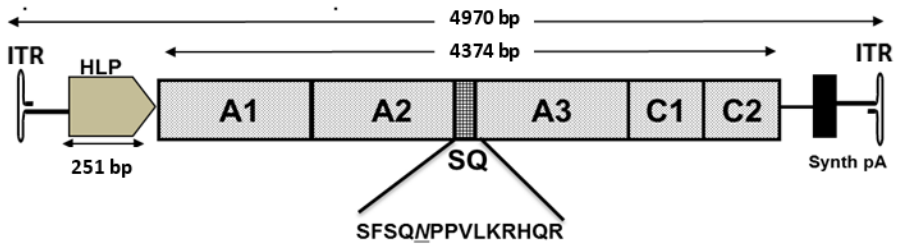
Figure 1. BMN 270 transgene construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

OBJECTIVES:

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-SQ-codop-hFVIII) vector.
- To determine the dose of AAV5-SQ-codop-hFVIII required to achieve expression of 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 rAAV5-SQ-codop-hFVIII
- 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

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STUDY DESIGN AND PLAN:

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled sequentially into one of up to three cohorts according to dose level:

1. 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
2. 2E13 vg per kilogram, iv
3. 6E13 vg per kilogram, iv

Patients will be enrolled sequentially every 3 weeks or more between cohorts. Dose escalation may occur after a single patient has been safely dosed if the resulting FVIII activity at Week 3 is < 5 IU/dL. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the patient numbers exposed to subtherapeutic doses.

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity is ≥ 5 IU/dL, then the other patients of the dose group will be enrolled without waiting for 3 weeks between patients.

Patient 1 will be dosed by intravenous perfusion with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥ 5 IU/dL at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥ 5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

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For each dose, if the activity level reaches 5 IU/dL and no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Because patients develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a treatment option and these patients have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dose. Any safety signal will trigger a review of the data and possible additional analysis including mononuclear collection.

NUMBER OF PATIENTS PLANNED:

Up to 12 patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII levels have ever declined to 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitates for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if receiving on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, or no inhibitor on 2 consecutive occasions within the past 12 months using a modified Nijmegen Bethesda assay higher than 0.6 Bethesda Unit (BU)
6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

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1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition and AAV5 total antibodies
2. Evidence of detectable viral load of HIV
3. Significant liver dysfunction as defined by abnormal elevation of ALT (alanine transaminase) to 3 times the upper limit of normal, bilirubin, alkaline phosphatase, or an INR (international normalized ratio) of 1.4. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
4. Evidence of any bleeding disorder not related to Haemophilia A
5. Platelet count of $< 100 \times 10^9$/L

Creatinine $\geq$ 1.5 mg/dL

6. Liver cirrhosis of any etiology as assessed by liver ultrasound
7. Hepatitis B if surface antigen is positive
8. Hepatitis C if RNA is positive
9. Treatment with any IP within 30 days prior to the screening visit
10. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Each patient will receive a single injection of BMN 270 as an intravenous infusion. The volume of infusion will depend on the dose level.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

The study is open label with comparison of FVIII activity to baseline values. No reference therapy will be evaluated in this study.

DURATION OF TREATMENT:

BMN 270 is given as a single dose by intravenous perfusion.

CRITERIA FOR EVALUATION:

Safety:

The following safety outcome measurements will be assessed:

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- Incidence of adverse events (AEs), including serious AEs (SAEs)
- Change in clinical laboratory tests (serum chemistry and haematology)
- Change in vital signs
- Change in physical examination
- Vector shedding
- Liver function tests (LFT)

No major toxicity is expected based on preclinical studies in mice and monkeys. An asymptomatic transaminitis was observed at week 8-10 after administration with an AAV8-FIX, providing the rationale for the following surveillance plan. Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.

There will be a detailed monitoring plan for assessment of cellular and humoral responses to AAV5 capsid and FVIII protein.

Efficacy:

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

Secondary efficacy measures include assessing the impact of BMN 270 on the frequency of FVIII replacement therapy and on the number of bleeding episodes during the study. Patients will be asked to keep a patient diary to record the details in these areas.

Pharmacodynamics:

The FVIII antigen and activity level as measured by an ELISA (antigen level) and by one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by the FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of Cb (baseline concentration), Css (steady state concentration), Tss (time to steady state), C(t) and AUC(0-t) where t is 16 weeks.

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STATISTICAL METHODS:

The sample size is based upon clinical considerations and could detect a strong clinical efficacy signal. Up to 12 patients may be dosed in the study. The patients will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature but data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active FVIII at $\geq$ 5 IU/dL by chromogenic FXa assay and/or one-stage APTT assay between 1 to 16 weeks following study treatment. FVIII activity will be assessed weekly during the study period. We can only assess the true steady state of FVIII protein produced from BMN 270 after a minimum of 96 hours has elapsed since the last infusion of FVIII protein concentrates.

Analysis of neutralizing antibody response and other immunological parameters as well as viral shedding will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AAV	adeno-associated virus
ADL	activities of daily living
ADR	adverse drug reaction
AE	adverse event
ALT	alanine transaminase
APTT	activated partial thromboplastin time
BPV	BioMarin Pharmacovigilance
BU	Bethesda Unit
CFR	Code of Federal Regulations
CRA	clinical research associate
CRF	case report form
eCRF	electronic case report form
DMC	Data Monitoring Committee
ED	exposure days
EOSI	events of special interest
ETV	early termination visit
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	coagulation factor VIII
FXa	coagulation factor Xa
GCP	Good Clinical Practice
HA	Haemophilia A
hFVIII	human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
IB	investigator brochure
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	independent ethics committee

IND	Investigational New Drug (application)
INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IV	intravenous
LFT	liver funtion test
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no-observed-adverse-effect level
PD	pharmacodynamics
PEG	polyethylene glycol
PK	Pharmacokinetics
PRO	patient-reported outcome
rhFVIII	recombinant human FVIII protein
REB	research ethics board
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source data verification
vg	vector genomes

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" (from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 [ICH E6]).

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

BioMarin Pharmaceutical Inc. (hereafter referred to as BioMarin or the Sponsor) conducts its studies according to the highest ethical and scientific standards. The following sections articulate standards to which Investigators will be held accountable, as well as matters of compliance to document adherence to such standards.

5.1 Institutional Review Board or Independent Ethics Committee

Investigators are expected to interact with Ethics Committees (ECs) promptly, as required, during the course of the study. This includes, but is not limited to, providing appropriate documentation to support study initiation and maintaining appropriate flow of safety and other information during the course of the study and for study close-out activities. BioMarin (or designee) will assist Investigators with access to timely and accurate information and with assurance of prompt resolution of any queries.

Prior to initiating the study, the Investigator will obtain written confirmation that the institutional review board (IRB) or independent ethics committee (IEC) [for Canadian protocols, Research Ethics Board (REB)] is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/IEC/REB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Investigator will provide the IRB/IEC/REB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the patients, including all ICFs translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/IEC/REB confirming unconditional approval of the protocol, the ICF and all patient recruitment materials are obtained in writing by the Investigator and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study are made to the IRB/IEC/REB and BioMarin by the Investigator in accordance with applicable guidance documents and governmental regulations.

5.2 Ethical Conduct of Study

It is expected that Investigators understand and comply with the protocol. This includes, but is not limited to: establishing and meeting enrollment commitments, including providing eligible patients for study enrollment; adhering to diagnostic or other procedures as specified in the protocol; and assuring appropriate compliance with study treatment administration and accountability.

This study will be conducted in accordance with the following:

- European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, for studies conducted within any European country
- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (ICH E6)

Specifically, this study is based on adequately performed laboratory and animal experimentation. The study will be conducted under a protocol reviewed and approved by an IRB/IEC/REB and will be conducted by scientifically and medically qualified persons. The benefits of the study are in proportion to the risks. The rights and welfare of the patients will be respected and the investigators conducting the study do not find the hazards to outweigh the potential benefits. Each patient will provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Patient Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (Section 4.8), United States Code of Federal Regulations (CFR) 21 CFR §50, European Clinical Trial Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC, and other applicable local regulations, will be obtained for each patient prior to entering the patient into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB/IEC/REB approval. BioMarin and the IRB/IEC/REB must approve the documents before they are implemented. A copy of the approved ICF , and if applicable, a copy of the approved patient information sheet and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or designee prior to any study-specific procedures being performed.

The Investigator will provide copies of the signed ICF to each patient and will maintain the original in the record file of the patient.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

During administration of informed consent, expectations regarding participation in the study should be made clear to patients. Patients who are not willing and/or are not able to comply with all aspects of the study should not be encouraged to participate.

Prior to beginning the study, the Investigator at each site must provide to BioMarin or designee, a fully executed and signed US Food and Drug Administration (FDA) Form FDA 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of patients in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical research associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each patient as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

In multicenter studies, a Coordinating Investigator will be identified who will be responsible for study overview. The Coordinating Investigator will read the clinical study report (CSR) and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe. The identity of the Coordinating Investigator and a list of all Investigators participating in the study will be provided in the CSR.

Laboratory evaluations will be performed at the local laboratories associated with the study sites. Refer to the Laboratory Manual for more details.

7 INTRODUCTION

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin.Haematol.). It is caused by mutations in the factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. Clinical manifestations of severe FVIII deficiency are frequent unprovoked bleeding episodes in joints and soft tissues causing permanent disability and occasionally death mostly after brain haemorrhage. Treatment in Western (Berntorp, 2012, Haemophilia.) countries consists of intravenous injection of plasma derived or recombinant FVIII protein concentrates at the time of a bleed, or prophylactically, to prevent bleeding episodes. The short half-life for FVIII (~8-12 hours) necessitates frequent infusions and makes this treatment prohibitively expensive for the majority of the world's haemophilia A patients. These individuals develop debilitating arthropathy and have a substantially increased risk of death from haemorrhage in life (Stonebraker, 2010, Haemophilia.). Chemical modification or bioengineering of FVIII may improve half-life to around 20 hours (Pipe, 2010, Blood). However, these long acting FVIII variants do not eliminate the need for lifelong FVIII protein administration or problems of FVIII inhibitor formation, which occurs in 30% of patients on standard FVIII replacement therapy (Nathwani, 1992, Baillieres Clin.Haematol.).

Gene therapy offers the potential of a cure through continuous endogenous production of human FVIII following a single administration of vector. Haemophilia A is well suited for this approach because its clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in low amounts (100-200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential and a modest increase in the level of FVIII (a plasma level of 2ng/ml protein leads to a 1% expression) can ameliorate the severe phenotype (Srivastava, 2012, Haemophilia); thus the therapeutic goal for gene therapy is a modest increase in hFVIII. Finally, the consequences of gene transfer can be assessed using simple quantitative rather than qualitative endpoints that can be easily assayed in most clinical laboratories.

BMN 270 contains the cDNA for the B-domain deleted SQ FVIII with a liver-specific HLP transcription promoter. The expression cassette is inserted between AAV2 ITRs and this genome is packaged in the AAV5 capsid. A comprehensive review of BMN 270 is contained in the IB supplied by BioMarin. Investigators are to review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program supports a single IV administration of BMN 270, the planned clinical route of administration, for the treatment of Haemophilia A in male patients. This nonclinical program took into account the guidelines and reflection papers for gene therapy medicinal products under EMA Advanced Therapies. The primary pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of IV BMN 270 were characterized in a series of single dose studies in species that were vector permissive and responsive to the transgene (normal CD-1 mouse, B and T cell deficient mouse model of haemophilia A (B6;129S-*F8*^{tm1Kaz}/J x B6.129S6-*Rag2*^{tm1Fwa} N12; FVIII KO x Rag2, and normal cynomolgus and rhesus monkeys). Some PD studies evaluated additional PK, immunogenicity and toxicity endpoints.

The primary PD of BMN 270 was assessed in a series of non-GLP single dose studies in a FVIII KO x Rag2 mouse model of severe Haemophilia A that recapitulates the change in coagulation observed in the human severe disease population without interfering cross species immunogenicity. PD activity and expression of hFVIII was assessed for durations up to 13-weeks in the FVIII KO x Rag2 mouse given dose range of 2E11 through 2E14 vector genomes (vg)/kg. Low levels of plasma hFVIII were observed in mice given 2E12 vg/kg after eight-weeks, post dose. Plasma hFVIII activity using bleeding time as an endpoint was evaluated in the FVIII KO x Rag2 mouse given up to 2E13 and 1E14 vg/kg BMN 270 at 8 weeks, post dose. BMN 270 related correction of bleeding times showed a dose relationship that was similar to that observed in mice given matched IU levels of Refacto®.

All cynomolgus monkeys used in this program were screened for neutralizing anti-AAV5 activity prior to study enrollment. PD activity and plasma hFVIII concentrations were evaluated in the cynomolgus monkey given up to 6E13 vg/kg BMN 270 for eight weeks. Peak expression of hFVIII was observed three to six weeks post administration with a subsequent decrease in expression presumably due to immunogenicity though not necessarily correlated to antibody titer.

The normal mouse was utilized in the GLP toxicology study to assess the safety profile of BMN 270 without the background of disease progression and in sufficient number per parameter. The GLP single dose toxicity study in normal CD-1 mice given 6E12 and 6E13 vg/kg with a 4 and 13-week follow up period monitored clinical pathology, defined potential target organs, immunogenicity, gene expression and activity. No changes in liver function were observed. In a single dose PD study in monkey given BMN 270, formation of anti-AAV5 total antibodies was observed at Week 8 with relatively low formation of anti-hFVIII total antibodies. No changes in liver function was observed.

The overall nonclinical safety profile includes formation of anti-AAV5 total antibodies with relatively lower formation of anti-hFVIII total antibodies. Immunogenicity against the AAV capsid and hFVIII is an anticipated finding for BMN 270 treatment and will be monitored in the nonclinical and clinical programs. Though not observed in the nonclinical species, liver toxicity utilizing clinical pathology parameters will be monitored in the clinical program based on previous literature. Cellular immune response will also be monitored.

Additionally, the BMN 270 transgene product has a final sequence matching that of the protein replacement treatment, Refacto® and therefore the safety profile of the BMN 270 transgene product is anticipated to be similar to Refacto® and have no unique FVIII-specific target organs of toxicity. The AAV5 capsid has not been modified and therefore no unique capsid-specific safety findings are anticipated. Because the biodistribution pattern of AAV5 after IV administration is consistent across species, BioMarin will confirm the nonclinical distribution pattern of BMN 270 during clinical development. Biodistribution of the AAV5 capsid has been documented to predominately target the liver with relatively lower levels of transduction observed in the lung, kidney, spleen, testes and blood compartment depending on the species and timing of administration. Liver targeted distribution of a similar AAV5 Factor IX gene therapy product was observed in the rhesus macaque monkey with low levels of vector genomes detected in spleen, kidney and testis (Paneda, 2009, Hum.Gene Ther.). Despite distribution to non-liver organs, no expression of Factor IX was detected in these tissues. Additionally, distribution to the testis was observed in another study in monkeys given an AAV5-codon-optimized human porphobilinogen deaminase; however, vector genomes in the semen were only transiently observed within one week but not thereafter (Paneda, 2013, Hum.Gene Ther.).

Both normal and disease model mice and a limited number of monkeys were utilized to establish proof of concept, species scaling and dose response in order to select the first-in-human (FIH) dose of 6E12 vg/kg. This dose represents > 30-fold safety factor from the no observed adverse effect level (NOAEL) in enabling nonclinical studies.

7.2 Previous Clinical Studies

This is the first clinical study for BMN 270.

BioMarin's program for haemophilia A builds on previous clinical trials of AAV-mediated gene transfer for a related condition, haemophilia B, that were conducted with an AAV2 vector (Manno, 2003, Blood) and an AAV8 vector (Nathwani, 2011, N.Eng.J.Med.; Nathwani, 2014, N.Eng.J.Med.). The large size of the FVIII cDNA was shortened and a preclinical validation of this approach was performed with an AAV8 vector (McIntosh, 2013, Blood).

AAV serotype 5 is also used in clinical trials and has shown good safety in a recent phase 1 study for treatment of Acute Intermittent Porphyria (D'Avola. 2014, J Hepatology).

AAV using other serotypes have been used in 105 clinical studies to date and no safety issue specific to the vector was ever reported.

7.3 Study Rationale

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin. Haematol). It is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be either inherited, due to a new mutation or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, but all are characterized by a defective coagulation process. . The clinical phenotype of HA patients is largely governed by the level of residual expression. Severe HA is classified as FVIII activity less than 1% of wild type (< 1 IU/dL), moderate disease comprises 1-5% of wild type activity and the mild form is 5-40% activity. The clinical manifestations of severe HA remain frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from haemorrhage when the brain is involved.

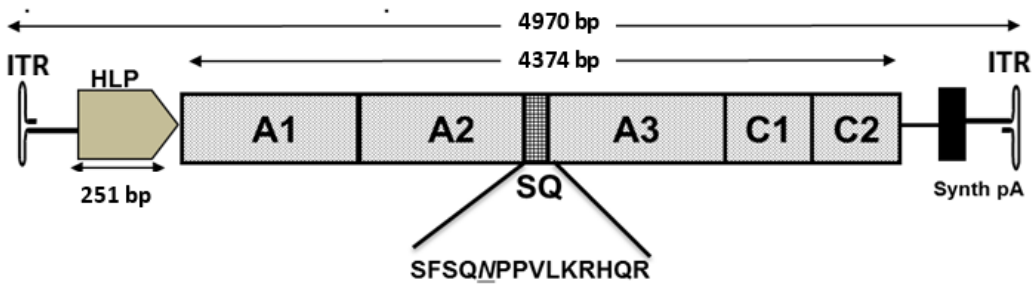
Treatment of severe HA presently consists of intravenous injection of plasma derived or recombinant FVIII protein (rhFVIII) concentrates both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for rhFVIII (under 24 hours for most approved products) necessitates frequent infusions, and although a major advance in the treatment of HA, it remains common for severe HA patients to continue to have multiple bleeding events on treatment (mean of 1 to 7 episodes/year with prophylaxis up to 30 to 50 for on demand treatment) (Nagel, 2011, Haemophilia). The consequence of multiple bleeding events is the development of an underlying pathology that contributes to debilitating multiple-joint arthropathy and substantially increased risk of death (Nagel, 2011, Haemophilia). Chemical modification (e.g. direct conjugation of polyethylene glycol (PEG) polymers) and bioengineering of FVIII (e.g. FVIII-Fc fusion proteins) improve half-life by approximately 50%, and thus, show promise in reduced dosing and maintaining activity levels above 1% trough (Stonebraker, 2010, Haemophilia), (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple infusions to maintain critical levels of FVIII activity in severe HA patients. There is therefore a strong unmet need for a fully preventive treatment of HA to give patients a FVIII level compatible with a normal and haemorrhage-free life.

Gene therapy offers the potential of disease-modifying therapy by continuous endogenous production of active FVIII following a single intravenous administration of a vector with the appropriate gene sequence. Haemophilia A is well suited for a gene replacement approach because clinical manifestations are attributable to the lack of a single gene product (FVIII) that circulates in minute amounts (200 ng/ml) in the plasma. Tightly regulated control of gene expression is not essential, and modest increases in the level of FVIII (any increase of the plasma level by 2 ng/ml induces an increase in activity of 1%) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous FVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using validated quantitative rather than qualitative endpoints that are easily assayed using established laboratory techniques.

Several different gene transfer strategies for FVIII replacement have been evaluated, but adeno-associated viral (AAV) vectors show the greatest promise (Pipe, 2010, Blood). They have an excellent and well defined safety profile, and can direct long term transgene expression with tropism for specific tissues such as the liver (Nathwani, 2005, Curr.Hematol Rep.) for serotypes 2, 5 and 8 among others). Indeed, an on-going gene therapy clinical trial for a related disorder, haemophilia B, has established that stable (>36 months) expression of human factor IX at levels that are sufficient for conversion of their bleeding phenotype from severe to moderate or mild is achievable following a single peripheral vein administration of AAV-8 vector(Nathwani, 2011, NEJM). Several participants in this trial have been able to discontinue factor prophylaxis without suffering spontaneous haemorrhages, even when they undertook activities that previously resulted in bleeding. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.). (Bainbridge, 2008, NEJM; Maguire, 2009, Lancet; Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses the SQ form of rhFVIII under the control of a liver-selective promoter (Figure 7.3.1).

Figure 7.3.1: BMN 270 Transgene Construct, ~5.0 kbp



BMN 270 will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue. The clinical study BMN 270-201 is a first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity, and whether these levels are sufficient to alter the clinical phenotype. The relationship of dose to safety will be correlated to the activity of hFVIII in patients with severe HA.

7.4 Summary of Overall Risks and Benefits

Safety will be assessed by adverse event reporting and clinical laboratory assessment. Based on prior human studies with peripheral injections of AAV vectors, there is no known safety risk of AAV gene therapy. However, a mild asymptomatic transaminitis was observed at week 8-10 after administration with an AAV8-FIX, providing the rationale for the following surveillance plan (Manno, 2006, Nature Med.). Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline. LFTs will be monitored every other month for the first year and then every three months for up to 5 years post-dose in the safety extension.

Dose selection: The benefit of this study can only be enhanced by selecting a starting dose that potentially could contribute to a better opportunity of observing a therapeutic effect, and therefore, reducing the possibility of a sub-therapeutic effect. With this consideration in mind, the starting dose 6E12 vg/kg was selected using overall data from the pre-clinical studies conducted in mice (normal and disease model, Rag2 -/- x FVIII -/-) and monkey.
A detectable pharmacological response based on activity was observed at 6E12 vg/kg.
A 10-fold safety margin based upon the NOAEL was observed in the GLP 13-week study in normal mouse at the highest dose administered. A 30-fold safety margin in an 8-week dose response study in disease model mice based on histology was observed at the highest dose administered. No BMN 270-related changes in clinical observations or chemistry was observed in the monkey at doses up to 6E13 vg/kg, a 10-fold safety margin after 8-weeks. Overall, no BMN 270-related findings, except expected formation of anti-AAV5 antibodies, were observed at the highest administered doses of 6E13, 2E14 and vg/kg in the normal mouse, disease model mouse and monkey, respectively.

Based on these key pre-clinical findings it can be suggested that the proposed starting dose of 6E12 vg/kg should not pose any risk or safety concerns to patients but may potentially benefit the patient therapeutically.

8 STUDY OBJECTIVES

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-SQ-codop-hFVIII) vector.
- To determine the dose of AAV5-SQ-codop-hFVIII required to achieve expression of hFVIII at or above 5% of normal activity (≥5 IU/dL) at 16 weeks after infusion. The kinetics, duration and magnitude of AAV-mediated hFVIII 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 hFVIII transgene product and AAV capsid proteins following systemic administration of rAAV5-SQ-codop-hFVIII
- 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

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Eligible patients will enter the dose escalation part of the study followed by a 16-week Post-Infusion Follow-Up period during which safety and efficacy assessments will be taken. After the primary endpoint analysis at 16 weeks, safety and efficacy will then be assessed for approximately 5 years.

Patients who provide written informed consent, meet the entry criteria definition of severe HA, and do not have transduction inhibition activity to AAV5 will be eligible to enroll in the study. Participants will be enrolled sequentially into one of up to three cohorts according to dose level:

1. 6E12 vector genomes [vg] per kilogram of body weight, given as a single intravenous dose (iv)
2. 2E13 vg per kilogram, iv
3. 6E13 vg per kilogram, iv

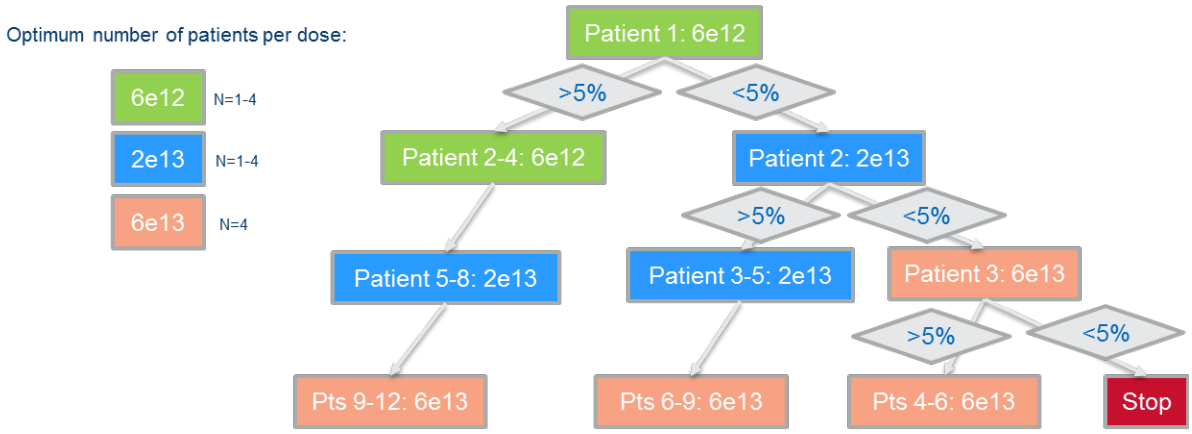
Patients will be enrolled sequentially every 3 weeks or more between cohorts. Dose escalation may occur after a single patient has been safely dosed if the resulting FVIII activity at Week 3 is < 5 IU/dL. Three weeks is expected to be the time the expression will be close to the maximum. This escalation paradigm is intended to minimize the patient numbers exposed to subtherapeutic doses.

The starting dose was based on the expression and safety of FVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from no observed adverse effect level (NOAEL) in non-human primates.

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

If the FVIII activity is ≥5 IU/dL, then the other patients of the dose group will be enrolled without waiting for 3 weeks between patients.

Figure 9.1.1: Flow Chart of Dose Escalation Scheme



Patient 1 will be dosed by intravenous perfusion with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥5 IU/dL (expressed as % in Figure 9.1.1) at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and if no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Because patients develop neutralizing immunity upon exposure to the capsid, re-challenge of vector is not a treatment option and these patients have effectively a single opportunity for therapy using this AAV capsid. Efficacy will be determined by FVIII activity at 16 weeks post dose. Safety will be evaluated for approximately 5 years after dose. Any safety signal will trigger a review of the data and possible additional analysis including mononuclear collection.

A summary of events and assessments are provided by visit in Table 9.1.1 for Screening, Baseline and BMN 270 Infusion, in Table 9.1.2 for Post-Infusion Follow-up, and in Table 9.1.3 for Safety Follow-up.

Table 9.1.1: Schedule of Events – Screening and Infusion

Assessment	Prior to BMN 270 Infusion		BMN 270 Infusion Visit (Day 1)
	Screening (Day -28 to Day -1)	**Baseline (Day -7 to Day -1)[i]**	
Informed consent	X		
Medical History	X		
Physical Exam[a]	X	X	X
Vital Signs	X		X
Assessment of Adverse Events and Concomitant Medications	X	X	X
Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)	X	X	
Electrocardiogram	X		
Chest X-ray	X		
Liver Ultrasound	X		
FVIII Treatment Washout[b]	X		
hFVIII Assays[c]	X	X	
AAV5 Assays[d]	X		
Screen for Hepatitis B, Hepatitis C, HIV[e]	X		
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[f]	X	X	
Mononuclear cell collection for CTL baseline		X	
PCR of vector genomes in blood, saliva, urine, semen, and stools		X	
Biomarker testing[g]	X		
Exploratory biomarker assessments[h]		X	
Quality of Life (QoL) assessment		X	
BMN 270 Infusion			X

[a] Complete physical examination should be done at Screening. Brief physical examination may be done at Baseline and at the BMN 270 Infusion Visit. Height and weight will be recorded at Screening only.

[b] Patients will have a washout period of 72 hours. If a bleeding occurs, another washout period of 72 hours will be performed. If another bleeding occurs during this second washout period, then the patient may be excluded from the study at the physician's discretion.

[c] Includes baseline hFVIII activity (chromogenic FXa and one-stage APTT assays), hFVIII coagulation activity exploratory assay, hFVIII inhibitor level (Bethesda assay with Nijmegen modification), hFVIII total antibody titer, and hFVIII antigen (ELISA). hFVIII assays should be assessed only when the patient has been off FVIII therapy for at least the previous 4 days.

[d] Screening (done during screening) and confirmation (at baseline) of AAV5 pre-existing immunity may include plasma samples for 3 assays (in vivo transduction inhibition, in vitro transduction inhibition and AAV5 total antibody assays).

[e] Patients with documented negative results within the last 30 days do not need to be retested.

[f] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[g] Includes HLA typing, FVIII mutation status, TNFα and IL10a single nucleotide polymorphisms.

[h] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

[i] Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

Table 9.1.2: Schedule of Events – Post-Infusion Follow-Up

	Follow-Up After BMN 270 Administration - Weeks*															
Assessment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Physical exam[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
hFVIII activity assays[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
hFVIII antibody titer	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Exploratory biomarker assessments[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
QoL assessment	X	X	X	X												X
AAV5 antibody titer	X							X								X
Testing for reactivation of hepatitis B and hepatitis C	X		X			X										
Mononuclear cell collection				X				X				X				X

* Visit windows are ±48 hours

[a] Brief physical examination should be done at all weekly visits except Week 16, where a complete physical examination should be performed. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included. Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

[c] Includes hFVIII activity level (APTT and FXa chromogenic assay), hFVIII activity confirmation (frozen plasma), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA).

[d] Collection to occur on Day 1, 3, 7 following BMN 270 infusion, and then weekly until 3 consecutive negative results are obtained

[e] Blood samples will be collected from subjects to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

Table 9.1.3: Schedule of Events – Safety Follow-Up

Assessment	Year 1 - Weeks*					Years 2-5*	ETV (early termination visit)
	20	28	36	44	52	Q3M	
Physical exam[a]	X	X	X	X	X	X	X
Assessment of Adverse Events and Concomitant Medications	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Blood chemistry, haematology, urine tests, coagulation screen, and CRP[b]	X	X	X	X	X	X	X
hFVIII activity assays[c]	X	X	X	X	X	X	X
AAV5 antibody titer	X	X	X	X	X	X	X
hFVIII antibody titer	X	X	X	X	X	X	X
Mononuclear Cell Collection (for determination of FVIII and Capsid specific CTL activity)	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, semen, and stools[d]	X	X	X	X	X	X	X
QoL assessment		X			X	X[e]	X

* Visit windows are ±72 hours

[a] Complete physical examination should be performed at Weeks 52 and every 52 weeks thereafter; brief physical exam may be performed at other study visits. Refer to Section 9.7.8.4.

[b] Refer to Table 9.7.8.2.1 for laboratory assessments to be included.

[c] Includes hFVIII activity level (one-stage APTT and chromogenic FXa assay), hFVIII activity confirmation (frozen plasma), Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level, hFVIII coagulation activity exploratory assay, and hFVIII antigen (ELISA).

[d] Sample testing during Safety Follow-Up is not required if 3 consecutive samples are cleared during the Post-Infusion Follow-Up period.

[e] QoL assessment during Years 2-5 of Safety Follow-up should be performed at every other visit (every 6 months) starting with the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period).

9.2 Discussion of Study Design, Including Choice of Control Group

Study 270-201 is designed to be a first-in-man, phase 1/2 open-label, dose escalation study in patients with severe haemophilia A. Three doses of BMN 270 will be evaluated and the dose escalation decision tree is summarized in Figure 9.1.1.

The decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

There will be no control group. Parameters for each patient will be compared to a pre-treatment assessment of safety (liver function) and efficacy (number of bleeds, use of replacement therapy).

As AAV based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

9.3 Selection of Study Population

Up to 12 severe haemophilia A patients may enroll into the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

Additional criteria for participation in the study are provided in Section 9.3.1 and Section 9.3.2.

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Males that are 18 years or older with established severe Haemophilia A as evidenced by their medical history. Patients will be considered as severe if their FVIII levels have ever declined to 1 IU/dL or less
2. Treated/exposed to FVIII concentrates or cryoprecipitates for a minimum of 150 exposure days (EDs)
3. Greater or equal to 12 bleeding episodes only if on on-demand therapy over the previous 12 months. Does not apply to patients on prophylaxis
4. Able to sign informed consent and comply with requirements of the trial
5. No history of inhibitor, or no inhibitor on 2 consecutive occasions within the past 12 months using a modified Nijmegen Bethesda assay higher than 0.6 Bethesda Unit (BU)

6. Sexually active patients must be willing to use an acceptable method of contraception such as double barrier, including hormonal contraception for at least 6 months post-treatment.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Detectable pre-existing immunity to the AAV5 capsid as measured by AAV5 transduction inhibition and AAV5 total antibodies
2. Evidence of detectable viral load of HIV
3. Significant liver dysfunction as defined by abnormal elevation of ALT (alanine transaminase) to 3 times the upper limit of normal, bilirubin, alkaline phosphatase, or an INR (international normalized ratio) of 1.4. Potential participants who have had a liver biopsy in the past 3 years are excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4
4. Evidence of any bleeding disorder not related to Haemophilia A
5. Platelet count of $< 100 \times 10^9$/L
6. Creatinine ≥ 1.5 mg/dL
7. Liver cirrhosis of any etiology as assessed by liver ultrasound
8. Hepatitis B if surface antigen is positive
9. Hepatitis C if RNA is positive
10. Treatment with any IP within 30 days prior to the screening visit
11. Any disease or condition at the physician's discretion that would prevent the patient from fully complying with the requirements of the study. The physician may exclude patients unwilling or unable to agree on not using alcohol for the 16-week period following the viral infusion.

9.3.3 Removal of Patients from Treatment or Assessment

Patients may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any patient who requests to be withdrawn. A patient's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the termination visit should be carried out.

BioMarin must be notified of all patient withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative

reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a patient from the study include, but are not limited to, the following:

- Patient requires medication or medical procedure prohibited by the protocol
- Patient does not adhere to study requirements specified in the protocol
- Patient was erroneously admitted into the study or does not meet entry criteria
- Patient is lost to follow-up

If a patient fails to return for scheduled visits, a documented effort must be made to determine the reason. If the patient cannot be reached by telephone, a certified letter should be sent to the patient requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each patient, before enrollment into the study, that for evaluation of study results, the patient's protected health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and IRB/IEC/REB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the US, from each patient. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the patient and the patient will be removed from the study.

9.3.3.1 Study Safety Evaluation Criteria

If any of the following events occur (except the persistence of AAV5) in a patient in the study who has received BMN 270 infusion, an extensive safety analysis will be performed and BioMarin and the Principal Investigator will discuss pursuing the trial or increasing the time before the next patient is enrolled.

- A 5-fold increase in ALT after BMN 270 administration
- The occurrence of other drug-related Grade III-IV toxicity, including liver failure and clinical hepatitis
- Drug-related Grade II toxicity that persists for at least 7 days
- The occurrence of neutralizing antibodies to hFVIII following BMN 270 infusion

- The persistent detection of the AAV vector genome in the semen of a participant more than 26 weeks after BMN 270 infusion, as discussed in Section 9.7.8.5
- The occurrence of a malignancy at any point after BMN 270 infusion that is assessed as possibly, probably, or definitely related to the study agent
- Any unexplained serious adverse event ≥ grade 4 assessed as at least possibly related to study drug

9.3.4 Patient Identification and Replacement of Patients

Each patient will be assigned a unique patient identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Patients who withdraw from the study after Week 16 will not be replaced.

9.3.5 Duration of Patient Participation

The duration of this study will be approximately 264 weeks. This includes 4 weeks of screening, 1 day of BMN 270 infusion, 16 weeks of Post-Infusion Follow-Up, and 244 weeks of Safety Follow-Up. A primary endpoint analysis on safety and efficacy will be performed at 16 weeks.

9.4 Treatments

BioMarin or its designee will provide the study site with study drug sufficient for study completion. BioMarin is responsible for shipping study drug to clinical sites.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product

9.4.2.1 Product Characteristics and Labeling

The study drug label includes the following information: lot number, required storage conditions, a precautionary statement, expiry date, study number, and BioMarin name and address. Labelling will be done according to country specific requirements.

9.4.3 Storage

At the study site, all IP must be stored under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be

stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

After a physical exam performed by the PI or designee, participants will be admitted on the day of BMN 270 infusion. An IV catheter will be inserted into a suitable peripheral vein (e.g. the median cubital vein) and flushed with saline. FVIII protein concentrate will not be given since venipuncture is a minimally invasive procedure in these individuals under ordinary conditions.

BMN 270 will be prepared and infused as a pure solution over a dose-dependent time. Prepared drug will be kept at room temperature prior to administration. An electric syringe will be used to infuse through an in-line, low protein binding 0.22 micron filter. BMN 270 will be infused through the catheter using an appropriate infusion pump while monitoring the vital signs (pulse, blood pressure, respiration rate and temperature) at 15 minute (± 5 minutes) intervals. The anticipated maximum interval from initiation of thawing of BMN 270 to completion of the infusion is 4 hours.

Following completion of the infusion, vital signs will be monitored hourly (± 5 minutes) for 6 hours and then every 2 hours (± 15 minutes) for 6 hours and then at 4 hour intervals. If the vital signs are stable the catheter will be removed 20-24 hours after the infusion. Hemostasis at the puncture site will be established by applying pressure according to standard protocol for infusing FVIII concentrates. Patients will remain hospitalized for 24 hours to observe for any immediate toxicity of the procedure. After 24 hours of hospitalization, participants will be discharged unless toxicity has been observed in which case hospitalization may be extended or transfer to an outpatient facility may occur based on the evaluation and judgment of the Principal Investigator after consultation with the Medical Monitor.

9.4.5 Method of Assigning Patients to Treatment Groups

Patients who meet all eligibility criteria (refer to Section 9.3.1 and Section 9.3.2) may be enrolled into the study. Approval by the Sponsor will be required prior to enrollment of each study patient. Upon their enrollment into the study, patients will be assigned a unique patient number and dose level by the Sponsor.

Patients are enrolled serially and their dose is defined in Section 9.4.6.2 below. Escalation is based on FVIII activity 3 weeks after injection. Patients may receive the next higher dose if the previous patient does not meet the activity criteria, or the same dose if the previous

patient meets the activity criteria. Up to 6 patients can have the same dose, for a total of 12 patients in this study.

9.4.6 Selection of Doses Used in the Study

The starting dose was based on the expression of hFVIII observed in nonclinical studies of mice and monkeys. The starting dose has a significant safety margin (10-fold) from NOAEL in non-human primates. The dose escalation is half-logs based.

9.4.6.1 Selection of Timing of Dose for Each Patient

A minimum of three weeks are required between patients, to allow for evaluation of safety and expression of hFVIII. After three weeks, depending on the activity level, the choice of the dose for the next patient will be made as described below.

9.4.6.2 Selection of Dose for Each Patient

The starting dose was determined by expression of hFVIII observed in mice and monkeys and a scale up factor based on previous experience, an improved purification process over previous trials (thus decreasing the risk of immune response), and a new evaluation of the titer (PCR performed after hairpin removal).

Approximately three weeks after an injection, the decision to escalate to the next dose level will be made based on the review of safety parameters and FVIII activity by the Sponsor and a panel of investigators participating in the study.

Patient 1 will be dosed with 6E12 vector genomes [vg] per kilogram of body weight. If the activity level does not reach ≥5 IU/dL at 21 days, then a higher dose (2E13 vg per kilogram) will be used for the next patient.

If the activity level does not reach ≥5 IU/dL after Patient 2, then the highest dose (6E13 vg per kilogram) will be used for the next patient.

For each dose, if the activity level reaches 5 IU/dL and if no safety issue is found, then up to four patients will receive this dose. If at any time activity levels reach 10 IU/dL or higher, no further dose escalation will take place, but additional patients will then be dosed at this dose level for a total of 6 patients per dose.

Refer to Figure 9.1.1 for a visual representation of the study design.

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter medications taken by a patient for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

The following medications are prohibited starting 30 days before Screening:

- Live vaccines
- Immunosuppressive agents

Haemophilia treatments are discussed in the washout section below (Section 9.4.8.1).

9.4.8.1 Factor VIII Washout

Patients will have a washout period of 72 hours. If a bleeding occurs, another washout period of 72 hours will be performed. If another bleeding occurs during this second washout period, then the patient may be excluded from the study at the physician's discretion.

9.4.8.2 Glucocorticoid Treatment of Elevated Hepatic Transaminases

During the Post-Infusion Follow-up Period, each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.

Reports of abnormal LFTs (defined as 1.5x the patient's baseline level) should be made to BioMarin within 24 hours of the lab value being available. If the LFTs are abnormal, treatment with prednisolone initiated at a dose of 60mg per day and then gradually tapered will be started immediately.

9.4.9 Treatment Compliance

Study drug will be administered to patients at the study site by a qualified professional. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study. Sites will be instructed to return all used and unused study drug containers.

9.5 Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) received, patients to whom IP is dispensed (patient-by-patient dose specific accounting), and IP lost or accidentally or deliberately destroyed. The Investigator or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the Investigator study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

There are no dietary or other protocol restrictions for this study.

9.7 Safety and Efficacy Variables

As this is a first-in-man study, the primary endpoint of safety will be assessed as explained in Section 9.7.8.

The primary efficacy measure will be to assess plasma FVIII activity. The efficacy goal of the study is to establish the dose relationship to FVIII activity ≥ 5 IU/dL at 16 weeks post BMN 270 administration.

9.7.1 Safety and Efficacy Measurements Assessed

The Schedule of Events (Table 9.1.1) describes the timing of required evaluations.

9.7.2 Primary Efficacy Variables

The primary efficacy measure will be the plasma FVIII activity monitored on a regular basis after BMN 270 dose. The efficacy goal of the protocol is to ascertain the dose range of BMN 270 required to convert severe HA patients to stable expression of FVIII at 5 IU/dL, i.e., a mild severity. This is associated in natural history studies with clinically superior long term outcomes (Nathwani, 1992, Baillieres Clin.Haematol.).

To measure the primary efficacy variable, the following assays will be used:

- FVIII activity (chromogenic FXa assay)
- FVIII activity by one-stage APTT (Activated Partial Thromboplastin Time)
- Exploratory FVIII activity assay

The FVIII activity level and the number of patients with FVIII activity ≥ 5 IU/dL will be summarized.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3. Details on performing the assays are included in the Laboratory Manual.

9.7.3 Secondary Efficacy Variables

Secondary efficacy measures will be the reduction in the frequency of factor replacement therapy, and reduction in the number of bleeding episodes. Patients will be asked at each study visit to report the use of factor replacement therapy and the number of bleeding episodes since the previous visit. This information will be captured on the patient's diary or other patient records.

Assessment of viral shedding at one week after BMN 270 infusion will also be considered an efficacy variable.

9.7.4 Immunogenicity

Immunogenicity assays will be performed on plasma. The assays will include detection of anti-capsid, and anti-FVIII total antibody, and determination of neutralizing antibodies against FVIII. Any increase in total antibody level, especially during the time period of 6-12 weeks, will be compared with FVIII activity measurements. Any abnormality of the liver parameters will lead to an immunogenicity assessment to determine anti-FVIII and capsid specific CTL. Anti-FVIII and capsid specific CTL assays will be performed on collected PBMCs.

Timing of assessment by these assays is provided in Table 9.1.2 and Table 9.1.3.

9.7.5 Pharmacodynamics

The hFVIII antigen and activity level will be measured by an ELISA (antigen) and by one-stage APTT and/or chromogenic FXa assay (activity). Individual antigen and activity plasma plots will be prepared to assess changes over 16 weeks. FVIII activity measurements will be used to determine PD parameters.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual values of C_b (baseline concentration), C_{ss} (steady state concentration), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The PD parameters and dose will be used to assess clinical pharmacology.

Sample collection times are indicated in Table 9.1.2 and Table 9.1.3.

9.7.6 Exploratory Biomarker Assessments

Blood samples will be collected from subjects at the time points indicated in Table 9.1.1 and Table 9.1.2 to evaluate biochemical, molecular, cellular, and genetic/genomic aspects relevant to Haemophilia A disease. The genetic/genomic testing on these exploratory samples is optional.

9.7.7 Quality of Life Assessment

A patient-reported outcome (PRO) questionnaire will be used to assess subject quality of life (QoL) during the study. Assessments will occur at the time points listed in the Schedules of Assessments.

Details regarding the QoL assessment will be included in the Study Reference Manual.

9.7.8 Safety Variables

Safety in this study will be determined from evaluation of AEs, clinical laboratory assessments with a particular attention to the liver function, vital signs assessments, physical examinations, and immunogenicity.

9.7.8.1 Adverse Events

The occurrence of AEs will be assessed continuously from the time the patient signs the ICF. The determination, evaluation and reporting of AEs will be performed as outlined in Section 10. Assessments of AEs will occur at the time points shown in Table 9.1.1.

9.7.8.2 Clinical Laboratory Assessments

Specific visits for obtaining clinical laboratory assessment samples are provided in Table 9.1.1. The scheduled clinical laboratory tests are listed in Table 9.7.8.2.1. Refer to the Study Reference Manual for instructions on obtaining and shipping samples.

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant. Each clinically significant laboratory result should be recorded as an adverse event.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.8.2.1: Clinical Laboratory Tests

Blood Chemistry	**Haematology**	**Urine Tests**	**Other**
Albumin	Haemoglobin	Appearance	CRP
Alkaline phosphatase	Haematocrit	Color	
ALT (SGPT)	WBC count	pH	**Coagulation Screen including:**
AST (SGOT)	RBC count	Specific gravity	APTT
Direct bilirubin	Platelet count	Ketones	PT/INR
Total bilirubin	Differential cell count	Protein	TT
BUN		Glucose	
Calcium		Bilirubin	
Chloride		Nitrite	
Total cholesterol		Urobilinogen	
CO_2		Haemoglobin	
Creatinine			
Glucose			
GGT			
LDH			
Phosphorus			
Potassium			
Total protein			
Sodium			
Uric acid			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; WBC, white blood cell; TT, thrombin time; INR, international normalized ratio.

9.7.8.3 Liver function and Hepatitis Testing

Patients will be screened for evidence of previous hepatitis B or hepatitis C infection at Screening. If medical records showing no evidence of Hepatitis B or Hepatitis C infection from the previous 30 days are available, then the Screening tests do not need to be done.

Evidence of ongoing infection (positive surface antigen for hepatitis B or positive RNA testing for hepatitis C) will be an exclusion criteria. If these parameters are negative and therefore no active infection is detected, then the patients may be included if they were vaccinated (for hepatitis B) or cleared the infection (for hepatitis C) and their liver function is acceptable.

A liver ultrasound and liver function testing at Screening will identify any significant hepatic dysfunction, which is defined as:

- More than 3x the normal ALT level
- More than 3x the normal bilirubin level
- Alkaline phosphatase above the normal cut off.
- INR over 1.4.
- Thrombocytopoenia under 100 x 10^9/L

9.7.8.4 Vital Signs, Physical Examinations and Other Observations Related to Safety

Vital signs will include seated systolic and diastolic blood pressure, heart rate, respiration rate, and temperature. Any clinically significant change in vital signs will be recorded as an AE.

Systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature will be assessed at Screening, Baseline, and at the beginning of each visit during the Post-Infusion Follow-Up and Safety Follow-Up periods. On the day of the BMN 270 Infusion, vital signs will be monitored hourly for 6 hours and then every 2 hours for 6 hours and then at 4 hour intervals.

A complete physical examination is necessary during Screening/Baseline; thereafter, brief physical examinations may be performed at the discretion of the investigator based on the patient's clinical condition. Particular attention should be given to signs of bleeding, as well as assessing possible hemarthroses.

A complete physical examination will include general appearance (head, eyes, ears, nose, and throat), cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, genitourinary, musculoskeletal, and neurologic systems (level of consciousness, speech, language, cranial nerves, motor strength, motor tone, abnormal movements, reflexes, upper extremity sensation, lower extremity sensations, gait, Romberg, nystagmus, and coordination).

A brief physical examination will include general appearance, cardiovascular, respiratory, neurologic, and gastrointestinal assessments.

Weight and height will be recorded at Screening only.

9.7.8.5 Viral Shedding

Viral shedding has been extensively studied in all similar clinical trials performed to date. (Appendix 1; Appendix 2; Schenk-Braat, 2007, J Gene Med; Croteau, 2004, Ann Occup Hyg). It has been constantly shown that the vector was not detectable anymore at 40 days, with its persistence in semen for 16 weeks in the seminal fluid but not in motile sperm (Manno, 2006, Nature Med.). In addition, it has always involved only an extremely small fraction of the injected dose.

Viral shedding will also be extensively studied in the present clinical trial, every week up to 16 weeks, then every 4 weeks to one year, then every three months. During the Post-Infusion Follow-Up period, patients will undergo testing of various bodily fluids to look for evidence of viral shedding for possible viral transmission. Bodily fluids will be tested by PCR at the time points indicated in Table 9.1.2 and Table 9.1.3. Fluids tested will include:

- Blood
- Saliva
- Semen
- Urine
- Stool

Details for sample collection and storage are provided in the Laboratory Manual.

10 REPORTING ADVERSE EVENTS

10.1 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs; measurement of protocol-specified haematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-defined events of special interest that are deemed critical to the safety evaluation of the study drug.

10.1.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a patient administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the patient that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study-drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Bleeding events that are normal events of haemophilia (ie, bleeding events which occur only because the patient is a haemophiliac) should not be recorded as AEs but will instead be captured in patient diaries. Bleeding events that occur where a normal (ie, non-haemophiliac) patient would bleed, such as bleeding as a result of trauma, should be recorded as adverse events. All bleeding events which meet criteria for being serious should be reported as serious adverse events (SAEs) whether or not they are bleeding events that are normal sequelae of haemophilia.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal
- Is life threatening

  Note: Life-threatening refers to an event that places the patient at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization. Hospitalization for less than 24 hours will not be considered to be an SAE. Hospitalization solely for the purpose of insertion of an in-dwelling IV catheter (such as a Port-a-Cath or other brand) for administration of study drug will not be considered an SAE.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect in the child or fetus of a patient exposed to IP prior to conception or during pregnancy
- Is an important medical event or reaction – that, based on medical judgment, may jeopardize the patient or require intervention to prevent one of the above consequences (e.g. anaphylaxis)

All adverse events that do not meet any of the criteria for SAEs should be regarded as non-serious AEs.

10.2.1 Events of Special Interest (EOSI)

The following EOSI need to be reported to the Sponsor within 24 hours of site awareness, irrespective of seriousness, severity or causality:

- Elevation of liver enzymes triggering a corticosteroid treatment

10.3 Methods and Timing for Capturing and Assessing Safety Parameters

10.3.1 Adverse Event Reporting Period

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, the reporting period for all non-serious AEs and SAEs begins and continues for approximately 5 years or until study discontinuation/termination, whichever is longer. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

10.3.2 Eliciting Adverse Events

Investigators will seek information on AEs and SAEs and EOSI, at each patient contact by specific questioning and, as appropriate, by examination. Information on all AEs and SAEs and EOSI, should be recorded in the patient's medical record and on the AE Case Report Form (eCRF).

10.3.3 Assessment of Seriousness, Severity, and Causality

The Investigator responsible for the care of the patient or qualified designee will assess AEs for severity, relationship to study drug, and seriousness (refer to Section 10.2 for SAE definitions). These assessments should be made by a study clinician with the training and authority to make a diagnosis (eg, MD/DO, physician's assistant, nurse practitioner, or DDS).

10.3.3.1 Seriousness

The investigator will assess if an AE should be classified as "serious" based on the seriousness criteria enumerated in Section 10.2. Seriousness serves as a guide for defining regulatory reporting obligations.

10.3.3.2 Severity

Severity (as in mild, moderate, or severe headache) is not equivalent to seriousness, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. The severity of each will be assessed using the defined categories in Table 10.3.3.2.1.

The Investigator will determine the severity of each AE and SAE and EOSI using the NCI CTCAE v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Table 10.3.3.2.1: Adverse Event Grading (Severity) Scale

Grade	Description	
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]	
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]	
4	Life threatening or debilitating: consequences; urgent intervention indicated	Grade 4 and 5 AEs should always be reported as SAEs
5	Death related to AE	

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

10.3.3.3 Causality

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF. To ensure consistency of causality assessments, Investigators should apply the guidance in Table 10.3.3.3.1.

Table 10.3.3.3.1: Causality Attribution Guidance

Relationship	Description
Not Related	• Exposure to the IP has not occurred OR • The administration of the IP and the occurrence of the AE are not reasonably related in time OR • The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts, evidence, or arguments to suggest a causal relationship to the IP.
Related	• The administration of the IP and the occurrence of the AE are reasonably related in time AND • The AE could possibly be explained by factors or causes other than exposure to the IP **OR** • The administration of IP and the occurrence of the AE are reasonably related in time AND • The AE is more likely explained by exposure to the IP than by other factors or causes.

Factors suggestive of a causal relationship could include (but are not limited to):

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the Investigator's assessment of causality for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

10.4 Procedures for Recording Adverse Events

10.4.1 Recording Adverse Events on a eCRF

Investigators should use precise medical terminology when recording AEs or SAEs on the AE eCRF. Avoid colloquialisms and abbreviations.

Record only one diagnosis, sign, or symptom per event field on the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the AE eCRF, using MedDRA (Medical Dictionary for Regulatory Activities) terminology.

10.4.1.1 Diagnosis versus Signs and Symptoms

Using accepted medical terminology, enter the diagnosis (if known). If not known, enter sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, then this diagnosis should be entered on the AE eCRF, replacing the original entries where appropriate.

10.4.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the AE eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the AE eCRF. For example, if severe haemorrhage leads to renal failure, both events should be recorded separately on the AE eCRF.

10.4.1.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation time points. Such an event should be recorded only once on the AE eCRF unless its severity increases or decreases (in which case it should be recorded again on the AE eCRF).

A recurrent AE is one that occurs and resolves between patient evaluation time points, but then subsequently recurs. All recurrences of the AE should be recorded on the AE eCRF.

10.4.1.4 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the AE eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the AE eCRF.

A clinical laboratory abnormality should be documented as AE if **any** of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity

- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

For purposes of this study, laboratory tests showing a decreased level of hFVIII activity should not be reported as adverse events unless signs of worsening are observed.

10.4.1.5 Pre-existing Conditions

A pre-existing condition is one that is present prior to administration of BMN 270. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study **only** if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, *more frequent* headaches).

10.4.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.4.1.5). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.4.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.4.1.8 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.3.1), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the AE eCRF.

10.4.1.9 Pregnancy

Although not an AE per se, pregnancy in the partner of a patient taking trial medication should be reported as an SAE to facilitate outcome monitoring by the Sponsor. Pregnancy in partner should be reported during the period up to 5 years after viral infusion.

Pregnancy in partner should be reported within 24 hours of the site becoming aware of the pregnancy by faxing the Pregnancy Form in the study reference materials to BPV. In addition, pregnancy in a patient is also reported on the End of Study eCRF. The Investigator must make every effort to follow the patient through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study patient, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the AE eCRF, and expeditiously reported to the Sponsor as an SAE.

10.5 Reporting Requirements

10.5.1 Expedited Reporting Requirements

All SAEs and EOSI that occur during the course of the AE Reporting Period (refer to Section 10.3.1), whether or not considered related to study drug, must be reported by entering the information in the AE eCRF and submitting to BioMarin Pharmacovigilance (BPV)

within 24 hours of the site becoming aware of the event. Investigators should not wait to collect information that fully documents the event before notifying BPV of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.

If the EDC is unavailable, all SAEs should be reported to BPV by completing the SAE Report Form and faxing or emailing the completed form to BPV within 24 hours of the site becoming aware of the event. Once the EDC is available, the information should be entered in the AE eCRF.

The reporting period for SAEs begins after informed consent is obtained and the first administration of study drug is given. It continues for approximately 5 years or until study discontinuation/termination, whichever is longer.

10.5.2 Ethics Committee Reporting Requirements

Reporting of SAEs to the Independent Ethics Committee (IEC) will be done in compliance with the standard operating procedures and policies of the IEC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IEC was properly and promptly notified as required.

10.6 Follow-up of Patients after Adverse Events

The Investigator should follow all unresolved AEs and SAEs until the events are resolved or have stabilized, the patient is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) should be documented on the AE eCRF and submitted to BioMarin Pharmacovigilance and in the patient's medical record to facilitate source data verification.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.7 Post-Study Adverse Events

At the last scheduled visit, the Investigator should instruct each patient to report, to the Investigator and/or to BPV directly, any subsequent SAEs that the patient's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a patient has discontinued or terminated study participation, if the Investigator believes

that the death or SAE may have been related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a patient that participated in this study.

10.8 Urgent Safety Measures

The regulations governing clinical trials state that the Sponsor and Investigator are required to take appropriate urgent safety measures to protect patients against any immediate hazards that may affect the safety of patients, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the patients, the Sponsor and the Investigator shall take appropriate urgent safety measures to protect the patients against any immediate hazard. The Sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/IEC/REB is notified at the same time."

The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the Early Termination Visit (ETV). Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin within 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (eg, modified dose amount or frequency not defined in protocol)
- Lack of study scientific value, or detrimental study conduct or management
- Discovery that the quality or safety of the IP does not meet established safety requirements

10.8 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.

Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax: PI
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: PI
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
E-mail: PI

11 APPROPRIATENESS OF MEASUREMENTS

The measures of efficacy to be used in this study are standard, ie, widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the patient, the Investigator or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

Screening assessments will be performed within 28 days (± 14 days) of BMN 270 infusion while baseline assessments will take place a within 7 days of BMN 270 infusion (Day 1). Should the screening visit occur within 7 days of the drug infusion, physical examination, vital signs, blood chemistry, haematology, urine tests, coagulation screen, and CRP do not need to be repeated at Baseline.

The following procedures will be performed during the Screening Period:

- Full medical history, including haemophilia A history, Hepatitis B, Hepatitis C, and HIV. Patients with documented negative results within the last 30 days do not need to be retested.
- Complete Physical Exam (including height and weight)
- Vital Signs (systolic and diastolic blood pressure, heart rate, respiration rate, and temperature)
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)
- Electrocardiogram
- Chest X-ray
- Liver Ultrasound
- FVIII Treatment Washout (refer to Section 9.4.8.1)
- Samples for hFVIII Assays
    - Baseline hFVIII activity – chromogenic FXa (plasma) (at least 96 hours off therapy)
    - Baseline hFVIII activity level –one-stage APTT assay (at least 96 hours off therapy)
    - hFVIII coagulation activity exploratory assay
    - hFVIII inhibitor level (Bethesda assay with Nijmegen modification)

- hFVIII total antibody assay
- hFVIII antigen (ELISA)

- Blood sample for AAV5 Assays
  - AAV5 antibody titer
  - AAV5 transduction inhibition assays
- Screen for Hepatitis B, Hepatitis C, and HIV if required
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Blood samples for Biomarker testing (HLA typing, FVIII mutation status, TNFα and IL10a single nucleotide polymorphisms)

12.3 Baseline Visit

Baseline values will be recorded from 1 to 7 days prior to the treatment visit. The following procedures will be performed during the Baseline Period:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Documentation of bleeding episodes and FVIII usage (by either patient or clinical information)
- Samples for FVIII Assays
  - FVIII activity – chromogenic FXa (plasma) (at least 96 hours off therapy)
  - FVIII activity level –APTT One stage assay (at least 96 hours off therapy)
  - FVIII coagulation activity exploratory assay
  - FVIII inhibitor level (Bethesda assay with Nijmegen modification)
  - FVIII antibody assay
  - FVIII antigen (ELISA)
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- Mononuclear cell collection for CTL baseline
- PCR of vector genomes in blood, saliva, urine, semen, and stools
- Exploratory biomarker assessments
- QoL assessment

12.4 Treatment Visit/BMN 270 Infusion Visit (Day 1)

There will be one treatment visit for each patient. Patients will be hospitalized for 24 hours for the BMN 270 Infusion Visit. The following procedures will be performed during the BMN 270 Infusion Visit:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- BMN 270 Infusion
- Vital Signs
    - Vital signs will be recorded prior to BMN 270 infusion and then every 15 minutes (± 5 minutes) during BMN 270 infusion. Following infusion, vital signs will be monitored every 1 hour (± 5 minutes) for the first 6 hours, every 2 hours (± 15 minutes) for the next 6 hours, and then at 4 hour (± 15 minutes) intervals for the remainder of the patient's hospitalization.

12.5 BMN 270 Infusion Follow-Up Visits

After BMN 270 has been infused, patients will return to the study site every week (± 48 hours) for 16 weeks, when the following procedures will be completed:

12.5.1 Every Visit

Every week (Weeks 1 through 16), the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
    - Each patient will have comprehensive surveillance plan monitoring LFTs once a week from dose to completion of Week 6, 3 times per week (every other day excluding weekends) from Weeks 7 to 12, and once a week from Weeks 13 to 16. If a patient's ALT is found to be greater than 1.5x baseline during these periods, then LFTs will be monitored three times per week or until the ALT returns to baseline.
- Samples for FVIII Assays
    - FVIII activity level (APTT)

- FVIII activity level (chromogenic FXa assay)
- FVIII coagulation activity exploratory assay
- Bethesda assay (with Nijmegen modification) for hFVIII inhibitor level
- FVIII activity confirmation (Frozen plasma)
- FVIII antigen (ELISA)

- FVIII antibody titer
- PCR of vector genomes in blood, saliva, urine, semen, and stools
  - Collection to occur on Day 1, 3, 7 following BMN 270 infusion, and then weekly until 3 consecutive negative results are obtained
- Exploratory biomarker assessments

12.5.2 Every 4 Weeks

Every 4 weeks (Weeks 4, 8, 12, and 16), the following procedure will be performed:

- Mononuclear cell collection

12.5.3 Weeks 1, 3, and 6

At Weeks 1, 3, and 6, the following procedure will be performed:

- Test for Hepatitis B and Hepatitis C reactivation

12.5.4 Weeks 1, 8, and 16

At Weeks 1, 8, and 16, the following procedure will be performed:

- AAV5 antibody titer

12.5.5 Weeks 1, 2, 3, 4, and 16

At Weeks 1, 2, 3, 4, and 16, the following procedure will be performed:

- QoL assessment

12.6 Safety Follow-Up – Year 1

After the 16 weekly infusion follow-up visits are complete, subjects will return to the study site at Weeks 20, 28, 36, 44, and 52 (± 48 hours), when the following procedures will be completed:

12.6.1 Every Visit

At Weeks 20, 28, 36, 44, and 52, the following procedures will be performed:

- Physical exam
- Assessment of Adverse Events and Concomitant Medications
- Vital Signs
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.

12.6.2 Weeks 28 and 52

At Weeks 28 and 52, the following procedure will be performed:

- QoL assessment

12.7 Safety Follow-Up – Years 2-5

During Years 2-5 of Safety Follow-up, patients will be assessed every 3 months (± 72 hours). At these times, the following procedures will be completed:

12.7.1 Every Visit

Every 3 months (± 72 hours), the following procedures will be performed:

- Physical exam

    - Complete Physical Exam will be performed every 52 weeks; Brief Physical Exams may be performed at other visits.
- Assessment of Adverse Events and Concomitant Medications
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (FXa chromogenic assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing during Safety Follow-Up is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs

12.7.2 Every Other Visit (Every 6 Months)

Every six months starting at the Week 78 visit (ie, 26 weeks after the Week 52 visit at the end of Year 1 of the Safety Follow-up period), the following procedure will be performed:

- QOL assessment

12.8 Early Termination Visit

The Early Termination visit will occur on the date the patient withdraws from the study, even if the date does not correspond to a protocol-specific visit.

If a patient leaves the study prior to the Week 270 visit, the patient will be asked to return to the study site and complete an Early Termination visit. At the Early Termination visit, as many of the following assessments as possible should be done:

- Physical exam

- Assessment of Adverse Events and Concomitant Medications
- Blood chemistry, haematology, urine tests, coagulation screen, and CRP (refer to Table 9.7.8.2.1)
- FVIII Assays
    - FVIII activity level (APTT)
    - FVIII activity level (chromogenic FXa assay)
    - FVIII coagulation activity exploratory assay
    - Bethesda assay (with Nijmegen modification) for FVIII inhibitor level
    - FVIII activity confirmation (Frozen plasma)
    - FVIII antigen (ELISA)
- AAV5 antibody titer
- FVIII antibody titer
- Mononuclear cell collection
- PCR of vector genomes in blood, saliva, urine, semen, and stools
    - Sample testing at the ETV is not required if 3 consecutive samples are clear during the Post-Infusion Follow-Up period.
- Vital Signs
- QOL assessment

12.9 End of Study

The study will end after the last patient completes the last Safety Follow-Up visit (Week 270). BioMarin reserves the right to discontinue the study any time for clinical or administrative reasons and to discontinue participation of an individual investigator or site for clinical or administrative reasons, including, but not limited to, poor enrollment or noncompliance with procedures of the protocol or GCP. In addition, the study may be terminated if, in the opinion of BioMarin, the safety of the study patients may be compromised.

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

The statistical analysis plan (SAP) will provide additional details on the planned statistical analysis. Unless otherwise stated, all analyses will be performed using SAS.

14.1.1 Interim Analyses

No formal interim analysis is planned. An analysis of the primary endpoint will be done following all patients having completed the study assessments through the end of the Post-Infusion Follow-Up Period (Week 16).

14.1.2 Procedures for Accounting for Missing, Unused and Spurious Data

Missing data will not be imputed.

14.2 Primary and Secondary Efficacy Analysis

The patients will be followed in a longitudinal manner and all the relevant information will be collected. The analysis of the data will be descriptive in nature. Descriptive statistics include the number of patients, mean, median, standard deviation, minimum, and maximum for continuous variables and count and percent for categorical variables. Efficacy variables will be summarized by dose cohort at each time point. Relationship between dose and efficacy results will be examined.

Data collected in a longitudinal manner may be analyzed using longitudinal methods such as mixed effect model which takes into account the correlation among the observation taken at various time points within a participant. Efficacy is a secondary endpoint, defined as biologically active hFVIII at ≥ 5 IU/dL by chromogenic FXa and/or one-stage APTT assay between 1 to 16 weeks following study treatment. hFVIII activity will be assessed weekly during the study period. We can only assess the true steady state of hFVIII protein produced from BMN 270 after a minimum of 96 hours has elapsed since the last infusion of FVIII protein concentrates.

14.3 Immunogenicity

Analysis of neutralizing antibody response and other immunological parameters will be primarily descriptive and involve both inter-participant and intra-participant comparisons across doses.

14.4 Pharmacodynamic Analyses

The FVIII antigen and activity level as measured by an Elisa (antigen level) and by a one-stage APTT and/or chromogenic FXa assay (activity level), will be used for plasma profiles; FVIII activity will be used to determine PD parameters. Traditional PK analysis is not applicable.

If supported by FVIII activity data, non-compartmental analysis and observation will be used to estimate individual PD parameter values of C_b (baseline), C_{ss} (steady state), T_{ss} (time to steady state), C(t) and AUC(0-t) where t is 16 weeks. Other parameters that may be used to describe individual FVIII protein and activity plasma profiles are C_{max} (maximum concentration) and C_{avg} (average concentration, AUC(0-t)/t). The parameters will be summarized using descriptive statistics.

14.5 Safety Analysis

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the Sponsor to assign system organ class and preferred term classification to events and diseases, based on the original terms entered on the CRF.

All AEs will be coded using MedDRA. The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-patient listing will be provided for those patients who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hepatitis is of interest, and the percentage of patients who report hepatitis will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. For each clinical laboratory test, descriptive statistics will be provided on Baseline as well as all subsequent visits. Descriptive statistics for physical examination results and vital signs will also be provided.

Detailed statistical methods will be provided in the SAP.

14.6 Determination of Sample Size

No formal sample size calculations based on statistical power were performed.

The sample size is determined based upon clinical consideration and could detect a strong clinical efficacy signal. Up to 12 patients may be dosed in the study; the actual number of patients will depend on the criteria for dose escalation. A total of 6 patients may be enrolled at a single dose level.

14.7 Analysis Populations

The Safety analysis population is defined as all enrolled patients who receive any study drug. The analysis of safety data will be performed on Safety Set.

The Full Analysis Set (FAS) is defined as all enrolled patients who receive study drug and have at least one Baseline and post-Baseline assessment. The FAS will be used for efficacy analysis.

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a patient's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB/IEC/REB must be sought, and the Investigator should inform BioMarin and the full IRB/IEC/REB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/IEC/REB must review and approve all protocol amendments. Protocol amendments that have an impact on patient risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC/REB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to patients, the ICF will be amended and approved by BioMarin and the IRB/IEC/REB, and all active patients must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE

There will be no DMC for this study, however a safety and efficacy evaluation board composed of the investigators and sponsor will be established. The primary responsibility of the board will be to assess patient's safety and efficacy data accumulated for a particular dose level and to make recommendations regarding dose escalation.

16 COMPENSATION, INSURANCE AND INDEMNITY

There will be no charge to study patients to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB/IEC/REB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study patient's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study patient has been injured by the IP or by procedures performed as part of the study. Any patient who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The patient should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the patient's health insurance company or other third party payer for the cost of this medical treatment. If the patient has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each patient. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application developed and validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the erroneous response and then selecting or entering the factual response. In compliance with ICH GCP Guidelines and 21 CFR Part 11, the system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and patient records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Patients must also allow access to their medical records, and patients will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to patient records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "Source Data Verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a patient's CRF casebook can be locked, data fields must be source data verified and all queries closed. Refer to the Study Monitoring Plan for details on which fields must be source data verified. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee,

will then set the status of the forms, visits, and the entire casebook to Locked. Upon completion of the CSR, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study patients, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify BioMarin immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The Investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The Investigator must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The Investigator /institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, patient charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the U.S. or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator /institution should retain patient identifiers for at least 15 years after the completion or discontinuation of the study. Patient files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should Investigator /institution be unable to continue maintenance of patient files for the full 15 years. It is the responsibility of BioMarin to inform the Investigator /institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/ethical_1author.html) and good publication practices (GPP).

21 REFERENCES

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Patients

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of patients.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential patients, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB/IEC/REB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human patients or others are reported to the IRB/IEC/REB. Additionally, he or she will not make any changes in the research without IRB/IEC/REB approval, except where necessary to eliminate apparent immediate hazards to human patients.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: 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

Protocol Number: 270-201

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the SpoPI

PI

Medical Monitor Signa Date

Printed name: PI MD, PhD, PI Clinical Sciences_____