BioMarin Pharmaceutical Inc.

AAV5-hFVIII-SQ (BMN 270)

Annex III A (non-confidential version)

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LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
AAV5-hFVIII-SQ	Single-stranded adeno-associated viral vector, serotype 5, encoding human factor VIII under control of the human HLP promoter.
Ad	Adenovirus
BIIC	Baculovirus infected insect cells
bp	Base pair
BSC	BioSafety Cabinet
BSL	Biosafety Level
CMO	Contract Manufacturing Organisation
cDNA	Complimentary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FVIII	Coagulation factor VIII
GCP	Good clinical practice
GMO	Genetically Modified Organism
HCC	Hepatocellular carcinomas
hFVIII	Human coagulation factor VIII
HPL	Human Liver-specific Promoter
HPV	Human papilloma virus
HSV	Herpes Simplex Virus
ITR	Inverted terminal repeats
IV	Intravenous
kb	Kilobases
kDa	kiloDaltons
LCA	Leber's Congenital Amaurosis
LOD	Limit of Detection
LOQ	Limit of Quantitation
MCB	Master Cell Bank
NHP	Non-human primate
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
Polh	polyhedron promoter
PPE	Personal Protective Equipment
QPCR	Quantitative polymerase chain reaction
rAAV	Recombinant adeno-associated viral vectors
RNA	Ribonucleic acid
SAE	Serious adverse event
SFM	Serum-free medium
ss	Single stranded
SUSAR	Suspected Unexpected Serious Adverse Reaction
vg	Vector genome

I. GENERAL INFORMATION

A. THE NAME AND ADDRESS OF THE APPLICANT

BioMarin Pharmaceutical Inc., 105 Digital Drive, Novato, CA 94949, USA

B. NAME, QUALIFICATIONS AND EXPERIENCE OF THE RESPONSIBLE SCIENTIST (S)

Prof. Dr. Kathelijne Peerlinck, Universitair Ziekenhuis Leuven
Dr. Alain Gadisseur, Universitair Ziekenhuis Antwerpen
Dr. Cedric Hermans, Cliniques Universitaires Saint-Luc, Bruxelles

C. THE TITLE OF THE PROJECT

Title: A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII in Hemophilia A Patients with Residual FVIII Levels ≤ 1 IU/dL Receiving Prophylactic FVIII Infusions (Protocol number: 270-301)

A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector– Mediated Gene Transfer of Human Factor VIII at a dose of 4E13 vg/kg in Hemophilia A Patients with Residual FVIII Levels ≤ 1 IU/dL Receiving Prophylactic FVIII Infusions (Protocol number: 270-302)

II. INFORMATION RELATING TO THE GMO

A. CHARACTERISTICS OF PARENTAL ORGANISM

1. SCIENTIFIC NAME

Adeno-associated virus (AAV).

2. TAXONOMY

Group: Group II (ss DNA)

Family: *Parvoviridae*

Subfamily: Parvovirinae

Genus: *Dependovirus*

Species: Adeno-associated virus/ serotype 5 (AAV 5)

3. OTHER NAMES (USUAL NAME, STRAIN NAME, ETC.)

The parental virus concerned in this application is a primate (human) AAV. There are several naturally occurring serotypes of human or non-human primate adeno-associated virus (denoted AAV1 to AAV11) and further variants yet to be fully characterised. The serotype of AAV is determined by the capsid of the virion, which is integral to the species / tissue tropism and infection efficiency of AAV.

The capsid proteins of the experimental vector (AAV5-hFVIII-SQ) are derived from the AAV5 serotype.

AAVs have also been isolated from other animal adenovirus stocks; these include avian AAV (Dawson *et al*., 1982; Yates *et al*., 1973), bovine AAV (Coria and Lehmkuhl, 1978; Luchsinger *et al*., 1970; Myrup *et al*., 1976), ovine AAV (Clarke *et al.*, 1979), caprine AAV (Arbetman *et al*., 2005) and snake AAV (Farkas *et al*., 2004).

4. PHENOTYPIC AND GENETIC MARKERS

Adeno-associated virus is a single-stranded DNA virus (Group II) which exists as a non-enveloped icosahedral virion with a diameter of approximately 25nm.

After entry into the host cell nucleus, AAV can follow either one of two distinct and interchangeable pathways of its life cycle: the lytic or the latent.

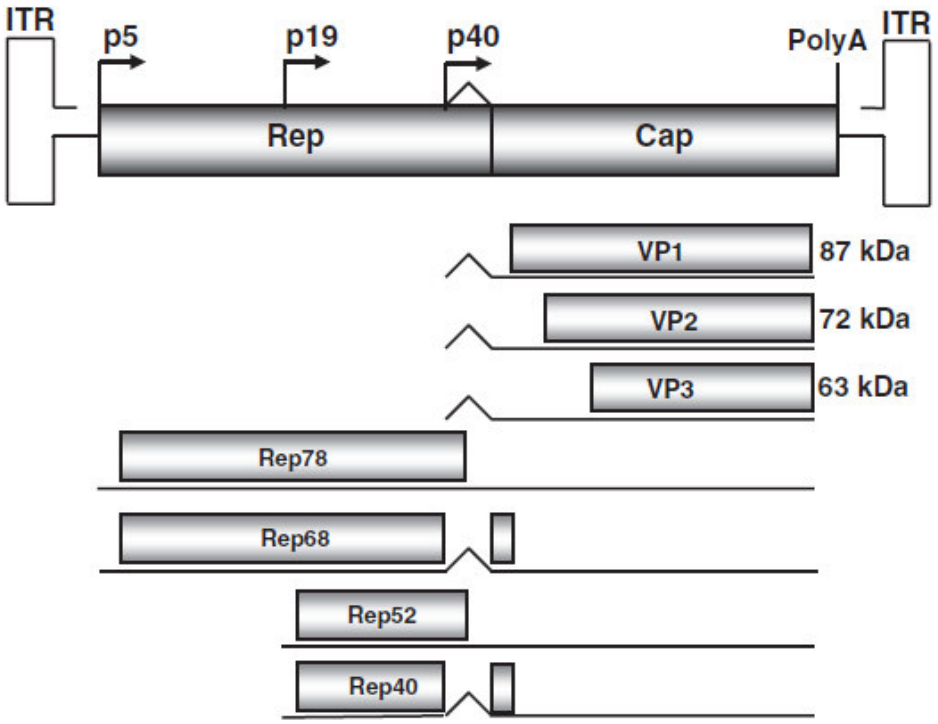
Productive (lytic) infection develops in cells co-infected with a helper virus such as adenovirus (Atchison *et al*, 1965; Henry, 1973), Human papilloma virus (HPV) (Hermonat, 1994; Su and Wu, 1996; Hermonat *et al*, 1997), vaccinia virus (Schlehofer *et al*, 1986) or herpes simplex virus (HSV) (Salo and Mayor, 1979; Buller *et al*, 1981) to help its replication.

In contrast, a latent state is established in host cells in the absence of a helper virus. Latency may manifest as a preferential integration of the virus genome into a region of roughly 2-kb on the long arm (19q13.3-qter) of human chromosome 19 (Kotin et al., 1990; Samulski et al., 1991) designated AAVS1 (Kotin et al., 1991). However, it has been demonstrated that only approximately 1 out of 1000 infectious units may integrate in cells in culture (Tenenbaum et al., 2003). Further work suggests that AAV DNA persists mainly as

circular double stranded episomes in human tissues (Schnepp et al., 2005).

The AAV genome is approximately 4.7 kilobases long and comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): *rep* and *cap* (Figure 1). The former is composed of four overlapping genes encoding Rep proteins required for DNA replication, and the latter contains overlapping nucleotide sequences coding for capsid proteins (VP1, VP2 and VP3) which interact together to form a capsid of an icosahedral symmetry.

Figure 1: Genome organisation of wild type adeno-associated viruses.



The single-stranded DNA genome of AAV. The inverted terminal repeats (ITRs) flank the two open reading frames *rep* and *cap*. The *rep* gene encodes four nonstructural proteins – Rep78, Rep68, Rep52, and Rep40. The *cap* gene encodes three structural proteins – VP1, VP2, and VP3. The location of the promoters, p5, p19, and p40 are depicted by arrows (Van Vliet *et al*., 2008)

The Inverted Terminal Repeat (ITR) sequences comprise 145 bases each and contain all *cis*-acting functions required for DNA replication and packaging, as well as for integration into, and subsequent excision and rescue from, the host cell genome in the latent state (Samulski *et al*., 1989).

There are several serotypes of adeno-associated virus (Reviewed in Wu *et al*., 2006). A serotype is, by definition, a newly isolated virus that does not efficiently cross-react with neutralizing sera specific for all other existing and characterized serotypes. Based on such, only AAV1–5 and AAV7–9 can be defined as true serotypes. Variants AAV6, 10, and 11 do not appear to fit into this definition, since the serology of AAV6 is almost identical to that of AAV1, and serological profiles of AAV10 and AAV11 are not well characterized.

The serotype of AAV is determined by the capsid of the virion, which is integral to the tissue tropism and infection efficiency of AAV. The AAV capsid is composed of

capsid protein subunits, VP1, VP2, and VP3, that are arranged in an icosahedral symmetry in a ratio of 1:1:10, with an estimated size of 3,900 kDa (Sonntag *et al.*, 2010). These structural elements are encoded by the *cap* gene of the AAV genome.

The *cap* proteins of the experimental vector (AAV5-hFVIII-SQ) are derived from the AAV5 serotype.

AAV serotypes 1 to 6 were isolated as contaminants in laboratory adenovirus stocks, with the exemption of AAV5, which was isolated from a human. AAV2 and AAV3 also are thought to be of human origin based on the prevalence of neutralizing antibodies in the human population. In contrast, AAV4 appears to have originated potentially in monkeys since antibodies against AAV4 are common in nonhuman primates. Whether AAV1 originated from human or nonhuman primates remains inconclusive. While antibodies to AAV1 were found in monkey sera, AAV1 viral genomes have been isolated from human tissues. AAV6 is likely a hybrid recombinant between AAV1 and AAV2, since the left ITR and p5 promoter regions are virtually identical to those of AAV2, while the rest of the genome is nearly identical to that of AAV1.

In the past few years, several novel AAV serotypes, including AAV7, AAV8 and AAV9, and over 100 AAV variants have been found in human or non-human primate tissues. The genomes of AAV7, AAV8 and AAV9 were identified after amplification from monkey tissue (Gao *et al.*, 2002; Gao *et al.*, 2004); however, isolation of these viruses has not yet been reported.

Some of these isolates show enhanced transduction in comparison with previously identified AAV serotypes in several tissue types. For example, AAV8, isolated in non-human primates, displays a propensity for liver transduction (Gao *et al.*, 2002).

5. DEGREE OF RELATEDNESS BETWEEN THE DONOR AND RECIPIENT ORGANISMS OR BETWEEN THE PARENTAL ORGANISMS.

Donor organism: Human

Recipient organism: Adeno-associated virus, serotype 5 (AAV5)

Donor and recipient organisms are not related although AAV5 can infect humans (refer to Section 8).

6. DESCRIPTION OF IDENTIFICATION AND DETECTION TECHNIQUES

The presence of AAV may be detected in clinical samples in three ways:

1. Polymerase Chain Reaction (PCR). PCR can be used to detect vector genome sequences associated with AAV in a qualitative or quantitative manner, using primers specific for the *rep* or *cap* genes. Detection of a specific serotype, or any AAV-like sequence, as well as distinction between wild type AAV and recombinant AAV is possible, depending on the choice of primers. Note that the presence of vector genomes does not necessarily imply infectious virus particles.

2. Viral culture: Samples containing suspected infectious AAV particles may be cultured in vitro on a permissive cell line, in the presence of a helper virus.

3. AAV vector particles may be detected using Enzyme-Linked Immunosorbent Assay (ELISA) methods. These methods rely on the generation of specific antibodies to the vector capsid proteins, and can therefore be specific to an individual serotype,

or cross-react with several AAV serotypes. Detection of vector capsid particles does not necessarily imply infectious virus particles.

7. SENSITIVITY, RELIABILITY (IN QUANTITATIVE TERMS) AND SPECIFICITY OF DETECTION AND IDENTIFICATION TECHNIQUES

The sensitivity/ specificity of the three methods for detection of wild type AAV is not apparent in the scientific literature, likely due to a lack of clinical utility of diagnostic methods due to the apparent lack of pathogenicity of the virus. It is possible to estimate sensitivity of detection of wild type AAV based on what is known regarding recombinant AAV vectors; for example qPCR methods typically have a Limit of Detection (LOD) of 10 copies per sample, and a Limit of Quantitation (LOQ) of 100 copies per sample. Similarly, viral cultures (utilising a helper virus) may be able to detect a minimum 1 replication competent (wild type) AAV particles in 1010 viral genomes.

8. DESCRIPTION OF THE GEOGRAPHIC DISTRIBUTION AND OF THE NATURAL HABITAT OF THE ORGANISM INCLUDING INFORMATION ON NATURAL PREDATORS, PREYS, PARASITES AND COMPETITORS, SYMBIONTS AND HOSTS

The human adeno-associated virus (AAV) is a globally endemic infection of humans, as demonstrated by the cross-reactivity of antibodies in the population to one or more AAV serotypes. Seroconversion occurs during childhood and is usually concomitant with an adenovirus infection (Reviewed in Tenenbaum et al., 2003).

Antibodies against AAV have been variously reported to be present in between 46-96% of individuals studied. Tenenbaum et al. (2003) cites studies of adults in Belgium and the USA (85-90% seropositive), France (63%), Brazil (55%), Germany (50%), and Japan (46%).

In a study by Chirmule et al. (1999), antibodies to AAV were seen in 96% of the subjects (patients with cystic fibrosis and healthy subjects). Boutin et al. (2010) report the prevalence of IgG cross-reactivity to specific serotypes: AAV1 (67%), AAV2 (72%), AAV5 (40%), AAV6 (46%), AAV8 (38%) and AAV9 (47%). However, in the study by Boutin et al. (2010) neutralizing factor seroprevalance was: AAV2 (59%), AAV1 (50%), AAV8 (19%) and AAV5 (3.2%).

There are no known natural predators, preys, parasites, competitors or symbionts associated with AAV, although it does require helper functions of co-infecting viruses for replication in nature.

9. ORGANISMS WITH WHICH TRANSFER OF GENETIC MATERIAL IS KNOWN TO OCCUR UNDER NATURAL CONDITIONS

AAV is thought to be spread in nature via inhalation of aerosolized droplets, mucous membrane contact or ingestion.

DNA replication occurs in the cell nucleus during lytic cycle. In its latent state, DNA is maintained either as a stable episome or by integration into the host cell DNA. It is important to note that integration of wild type AAV genomes into host cell DNA, as seen in cell cultures, requires the trans-acting function of the AAV rep gene, but this is not present in vectors.

Primate (human) AAV serotypes are not known to actively transfer genetic material to organisms other than primates under natural conditions, although an absence of zoonosis

is not documented. AAV can replicate in cells of a different species when infected with AAV in vitro, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987).

Evolution of AAV viruses (like all viruses) is directed by spontaneous mutation or homologous recombination with other viruses of the same species, where such genetic modification confers a selective advantage.

Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus which is permissive in that species (triple-infection).

10. VERIFICATION OF THE GENETIC STABILITY OF THE ORGANISMS AND FACTORS AFFECTING IT

In general, DNA viruses have greater genetic stability than RNA viruses. DNA is more thermodynamically stable than RNA and DNA replication is a less error prone process than is replication of RNA. Genetic stability of AAV5-hFVIII-SQ is supported by production under cGMP regulations, and verified by testing for purity, potency and composition.

Homologous recombination may occur if a host organism is infected with wild type AAV plus a helper virus and BMN 270 DP, which would require a triple infection.

11. PATHOLOGICAL, ECOLOGICAL AND PHYSIOLOGICAL TRAITS

(a) Classification of hazard according to existing Community rules concerning the protection of human health and/or the environment

The parental virus AAV is not pathogenic. The vector has had all viral genes removed and is assumed to also be non-pathogenic. The vector has fully wild-type AAV5 capsid proteins and the immune response is not expected to be different from that of the parental virus. The AAV meets the definition of biological agent of group 1 according to Directive 2000/54 / EC ("biological agent that is unlikely to cause human disease").

(b) Generation time in natural ecosystems, sexual and asexual reproductive cycle

Replication of AAV5 in an infected host can take from 24 to 48 hrs, but replication requires the co-infection with a helper virus. Note that the AAV rep and cap gene coding sequences were removed from AAV5-hFVIII-SQ vector so it cannot replicate even in the presence of a helper virus such as an adenovirus.

The only way that AAV5-hFVIII-SQ vector might be replicated would be in the presence of a helper adenovirus, such as adenovirus, and a wild type AAV to provide the transacting *rep* and *cap* genes. However, even this may be a very low likelihood as was tested by Afione et al (1996) in non-clinical studies that preceded the very first clinical trial of any AAV vector, in which an AAVCF vector was to be administered by airway delivery to cystic fibrosis patients. In the non-clinical studies, the vector was administered to the lungs of non-human primates. However, the vector could not be replicated or mobilized in the presence of both adenovirus and wild type AAV whether the latter two viruses were administered to the lung before or after the vector.

On the basis of the vector construct, it is considered that the BMN 270 vector construct has been appropriately engineered with characteristics such that the adeno-associated

viral vector has been sufficiently attenuated resulting in disablement of the vectors ability to infect, replicate or survive outside of humans.

(c) Information on survival, including seasonability and the ability to form survival structures

Wild type AAV survives in the environment as a persistent infection in the host vertebrate species or as a latent infection in the nucleus of some infected cells in culture, where it may remain inactive indefinitely, or be reactivated by helper virus giving rise to secretion of virus.

Due to its reliance on a helper virus for replication (typically adenovirus) AAV may be considered to exhibit seasonality. Temperate regions experience seasonal vagaries in the occurrence of adenoviral infections, with the highest incidences occurring in the autumn, winter, and early spring.

Outside of the host, non-lipid enveloped viruses such as AAV are resistant to low level disinfectants, survive well outside of the laboratory environment and can be easily transmitted via fomites. AAV particles are resistant to a wide pH range (pH 3-9) and can resist heating at 56^{o}C for 1 hour (Berns and Bohenzky, 1987). AAV does not form survival structures but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

It is destroyed by 0.5% sodium hypochlorite aqueous solutions and cleaning agents or hand-sanitizers that are effective against non-enveloped viruses.

(d) Pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms

There are three modified aspects of the vector that address pathogenicity; the removal of nearly all of the viral DNA, the maintenance of the native tropism by maintaining a wild type capsid and the insertion of the human gene FVIII. The removal of viral DNA, including all the *rep* and *cap* gene coding sequences, eliminates any viral virulence. The maintenance of the wild type capsid maintains wtAAV host range, tropism and absence of adverse immune responses due to AAV. The biologically active human FVIII-SQ protein expressed from the vector has been found to be well-tolerated in non-clinical and clinical studies with no vector-related clinically notable adverse events.

Depending on the Country, populations and studies published, approximately 3% to 40% of the human population in Europe is already seropositive for AAV5 (Boutin et al, 2010) and over 90% positive for one or several isotypes of AAV.

AAV5-hFVIII-SQ belongs to the type of AAV vector commonly referred to as a “gutted” AAV vector. As such, all of the viral gene-coding sequences have been removed. The only remaining sequences are two small, inverted terminal repeats (ITRs) that flank the transgene expression cassette and comprise a combined total of only 290 bases. The ITRs allow the vector genome to be packaged into vector capsids. Unlike the parent virus, the vector is not replication competent even in the presence of helper virus. The elimination of the viral DNA reduces the probability of homologous recombination with related viruses that could lead to variants of the GMO.

Like the parent organism, AAV5, the vector is non-pathogenic. The vector has had all viral genes removed and is assumed to also be non-pathogenic. The vector has fully wild-type capsid proteins and the immune response is not expected to be different from that of the

parental virus. Exposed individuals will likely seroconvert to an AAV5 antibody positive titre.

(e) Antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy

Antibiotics are not effective in the treatment of viral infection, nor does wild type AAV present specific resistance to antibiotics. The wild type virus does not contain any gene that confers resistance to known antibiotics.

(f) Involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.

Wild type AAV is not known to be involved in environmental processes. It does not respire and does not contribute to primary production or decomposition processes. In its virion form, it does not display any metabolic activity.

12. NATURE OF INDIGENOUS VECTORS

a) sequence: A high resolution crystal structure of wild type AAV5 has been reported (Walters, 2004, J Virol). The AAV5 capsid is composed of three capsid proteins (VP1, VP2 and VP3), grouped into 60 subunits per virion to form icosahedral shaped particles which are typically about 25 nanometers in diameter. Wild type AAV genomes are typically 4700 nucleotides in length.

b) frequency of mobilisation: The presence of natural mobile genetic elements such as transposons or plasmids related to AAV has not been reported.

c) specificity: AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV in vitro, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987). It is not known whether zoonosis occurs in nature.
The primary indigenous vector of primate (human) AAV serotypes is human beings. AAVs have also been isolated from other animal adenovirus stocks; these include avian AAV (Dawson *et al*., 1982; Yates *et al*., 1973), bovine AAV (Coria and Lehmkuhl, 1978; Luchsinger *et al*., 1970; Myrup *et al*., 1976), ovine AAV (Clarke *et al.*, 1979), caprine AAV (Arbetman *et al*., 2005) and snake AAV (Farkas *et al*., 2004).

d) presence of genes which confer resistance: The wild-type virus does not contain any gene that confers resistance to known antibiotics.

13. HISTORY OF PREVIOUS GENETIC MODIFICATIONS

The parent AAV5 is wild type. The cassette sequence codes for a B domain-deleted human coagulation factor VIII, which is under the control of a liver-specific promoter.

B. CHARACTERISTICS OF THE VECTOR

1. NATURE AND SOURCE OF THE VECTOR

The vector in this context comes from the plasmid, which is the source of the entire AAV5 vector (GMO) genome insert. A separate plasmid contains the viral rep and cap genes required for AAV5-hFVIII-SQ production.

2. SEQUENCE OF TRANSPOSONS, VECTORS AND OTHER NON-CODING GENETIC SEGMENTS USED TO CONSTRUCT THE GMO AND TO MAKE THE INTRODUCED VECTOR AND INSERT FUNCTION IN THE GMO

The BMN 270 vector genome contains ITRs at its 5'-end and 3'-end, a hybrid human liver-specific promoter (HLP), a B domain-deleted human factor VIII (hFVIII-SQ) gene, and a synthetic polyadenylation signal (SpA).

3. FREQUENCY OF MOBILISATION OF INSERTED VECTOR AND/OR GENETIC TRANSFER CAPABILITIES AND METHODS OF DETERMINATION

AAV5-hFVIII-SQ is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

Homologous recombination between AAV5-hFVIII-SQ and a wild type AAV could occur if both were present in the same cell. However, such recombination could only result in the exchange of the hFVIII expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV5-hFVIII-SQ (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, (as noted in Section A 10.b above) especially since the vector target cells (liver) are not the natural target cells of helper viruses. If it did occur, it would only result in the production of more wild type AAV and more AAV5-hFVIII-SQ vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining).

4. INFORMATION ON THE DEGREE TO WHICH THE VECTOR IS LIMITED TO THE DNA REQUIRED TO PERFORM THE INTENDED FUNCTION

The expression cassette is limited to the required elements designed to optimise expression of functional human coagulation Factor VIII in the liver.

The expression cassette is flanked by the inverted terminal repeats derived from AAV type 2. The remainder of the information is confidential and is not disclosed.

C. CHARACTERISTICS OF THE MODIFIED ORGANISM

1. INFORMATION RELATING TO THE GENETIC MODIFICATION

(a) Methods used for the modification;

The plasmids were constructed using standard molecular biological techniques for the precise excision and ligation of component elements using specific restriction enzymes followed by transduction and amplification in bacterial cells at each stage.

The remainder of the information is confidential and is not disclosed.

(b) Methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;

Sf9 cells are used for production of the AAV5-hFVIII-SQ vector. The ITRs are the only AAV-derived DNA sequences included in the vector genome and are required for its replication and encapsidation during production of the product.

(c) Description of the insert and/or vector construction;

BMN 270 is a recombinant, replication-incompetent, adeno-associated virus serotype 5 (AAV5) vector containing a DNA genome, AAV5-hFVIII-SQ

The ITRs are the only AAV-derived DNA sequences included in the vector genome and are required for its replication and encapsidation during production of the product.

(d) Purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function;

The sequence of the insert is limited to the DNA required to perform the intended function. Each lot of AAV5-hFVIII-SQ (containing the hFVIII expression cassette) is tested for identity by PCR to verify the integrity and identity of the insert. For the region in which sequence data was obtained, the sequenced sample was 100% identical to the expected sequence.

(e) Methods and criteria used for selection;

Antibiotics are not used in the manufacturing process used to construct the AAV5-hFVIII-SQ vector.

(f) Sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question with particular reference to any known harmful sequence.

Figure 2 illustrates the recombinant adeno-associated virus AAV5-hFVIII-SQ vector. The AAV5-hFVIII-SQ vector genome contains ITRs at its 5'-end and 3'-end, a hybrid human liver-specific promoter (HLP), a B domain-deleted human factor VIII (hFVIII-SQ) gene, and a synthetic polyadenylation signal (SpA).

Table 2 summarizes the AAV5-hFVIII-SQ elements.

Figure 3: Schematic of the AAV5-hFVIII-SQ vector genome

Legend: Note that schematic is not to scale; nt = nucleotides

Table 2: AAV5-hFVIII-SQ Components

Component	Function
DNA components	
AAV2 ITR	ITR of the most extensively studied AAV. Included in all AAV vectors used for clinical studies (145 nt).
Human liver promoter (HLP)	Regulates human liver-specific expression of neighboring gene (251 nt)
Kozak consensus sequence	Sequence located upstream of start codon in leader sequence is a part of known Kozak consensus sequence and enhances the translation in mammalian cells.
Leader sequence	N-terminal signal sequence for FVIII protein.
hFVIII-SQ gene	*hFVIII-SQ* gene encodes human FVIII-SQ protein. Amino acid sequences of A and C domains are identical to those of human FVIII.
SpA	Synthetic poly(A) signal including minimum sequence necessary for efficient polyadenylation

2. INFORMATION ON THE FINAL GMO

(a) Description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;

BMN 270 survivability is expected to be similar to AAV5 because the structure of the viral particle has not changed. That is, the capsid proteins are wild type AAV5 proteins and the vector genome is of similar size to the AAV5 genome. The entire viral genome has been removed except for the two small inverted terminal repeats (ITRs) comprising a total of only 290 bases and replaced with the hFVIII expression cassette. A new characteristic is that AAV5-hFVIII-SQ cannot replicate even in the presence of helper virus because the viral genome is absent.

Another new characteristic is that if AAV5-hFVIII-SQ enters a cell it cannot integrate into the genome because sequences responsible for integration have been removed. Integration of the AAV5-hFVIII-SQ genome has not yet been evaluated experimentally. Extensive studies with AAV2, AAV1, AAV8, and AAV5 vectors in rodents (Schnepp, 2003, J.Virol.), (Inagaki, 2008, J.Virol.), (Li, 2011, Blood), rabbits (Schnepp, 2006, Mol Ther), nonhuman primates (Nowrouzi, 2012, Mol.Ther.) and in human subjects who were administered Glybera (an approved gene therapy product) (Kaeppel, 2013, Nat.Med.) lead to the

estimation that the integration frequency of AAV vectors is several orders of magnitude lower than the spontaneous rate of mutation for human genomes (Cole, 1994, Mutat.Res.) so that the likelihood of insertional mutagenesis by AAV vectors is very low, although specific carcinogenicity studies have not been performed.

(b) Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;

The plasmid vector DNA present in AAV5-hFVIII-SQ is limited to only the intended hFVIII transgene expression cassette and the two small, viral inverted terminal repeats. The terminal repeats are each 145 nucleotides long.

(c) Stability of the organism in terms of genetic traits;

All tests for identity, purity and quality have confirmed the stability of AAV5-hFVIII-SQ. A potency assay verified that AAV5-hFVIII-SQ makes functional human factor VIII in vitro, and animal studies demonstrated that AAV5-hFVIII-SQ makes functional human FVIII in a dose dependent manner in vivo.

AAV5-hFVIII-SQ is not replication competent and has been tested for purity to demonstrate that no detectable replication-competent AAV is present.

(d) Rate and level of expression of the new genetic material. Method and sensitivity of measurement;

AAV5-hFVIII-SQ distributes to the target organ, liver, where its liver-specific promoter (HLP) drives expression of hFVIII-SQ. Plasma hFVIII-SQ protein or activity levels can be assessed by ELISA or Factor VIII activity assays, respectively. The remainder of the information is confidential and is not disclosed. .

(e) Activity of the expressed protein(s);

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 gene that codes for FVIII protein, an essential cofactor in the coagulation cascade. It is not a toxic or oncogenic protein.

(f) Description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector;

Polymerase Chain Reaction (PCR) can be used to detect vector genome sequences associated with AAV in a qualitative or quantitative manner, using primers specific for the rep or cap genes. Detection of a specific serotype, or any AAV-like sequence, as well as distinction between wild type AAV and recombinant AAV is possible, depending on the choice of primers. Note that the presence of vector genomes does not necessarily imply infectious virus particles.

(g) Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;

The primers and probe designed for the *FVIII-SQ* qPCR assay specifically detects a unique fragment of the codon-optimized human *FVIII-SQ* gene and not endogenous wild type *FVIII* or other genes.

(h) History of previous releases or uses of the GMO;

AAV5-hFVIII-SQ has been studied in an ongoing phase 1/2, dose-escalation study being performed in the UK, with a total of 15 males receiving a peripheral intravenous dose ranging from 6 x 10^{12} vg/kg to 6 x 10^{13} vg/kg administered to subjects at a central dosing facility. Similar to the experience from prior AAV-mediated gene transfer studies, no late, vector-related complications have been observed to-date (Pasi et al., 2017).

(i) Considerations for human health and animal health, as well as plant health:

(1) Toxic or allergenic effects of the GMOs and/or their metabolic products;

Neither wild type AAV nor the experimental vector BMN 270 is known to be pathogenic to humans.

AAV5-hFVIII-SQ is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

The possible effects of direct administration to patients are summarised below. In the case of transfer of vector to an unintended human recipient, the risks are expected to be considerably reduced, since the vector is not able to replicate and the 'dose' which may conceivably be transferred (from e.g. aerosol, splashing or fomites) will be orders of magnitude lower than that received by patients.

The possibility of inadvertent transfer of AAV has been directly examined (Croteau et *al.,* 2004). In this study an AAV vector was administered by nebulized aerosol to patients with cystic fibrosis and the exposure of health care workers involved in the vector administration was examined. Some low airborne vector particles were detected and the estimated dose to which the health care workers were potentially exposed was estimated to be 0.0006% of the administered dose of 10^{13} vg. No negative health effects were reported and the health care workers serologic status for AAV2 did not change. Nebulization might be considered a "worst case" for dissemination of the vector to others.

Potential risks of direct administration of AAV5-hFVIII-SQ to human haemophiliacs

The transgene of AAV5-hFVIII-SQ encodes a protein that is identical to the recombinant human coagulation Factor VIII (Refacto/ Xyntha), which is routinely used to treat haemophilia A. The subjects in the clinical trial will all have been treated/exposed to FVIII concentrates or cryoprecipitate and will be those who have not generated any antibody response to the protein. Based on data from infusion of recombinant FVIII in patients with haemophilia A, circulating FVIII levels as high as 100% of normal are not associated with ill-effects since the protein circulates as a zymogen (inactive precursor).

In the ongoing clinical trial of BMN 270 performed in the UK, FVIII activity levels above the normal range were observed in the 6E13 vg/kg cohort. These values have not been

associated with any clinical findings suggestive of a thrombotic event and have not required any medical intervention.

The long-term safety of recombinant AAV vectors in humans is unknown; however, AAV vectors have been delivered to many hundreds of human subjects to date, in trials for cystic fibrosis, rheumatoid arthritis, Leber's Congenital Amaurosis (LCA), α_1-antitrypsin deficiency, congestive heart failure, lipoprotein lipase deficiency, as well as haemophilia, and have been remarkably free of vector-related adverse events (Mingozzi and High, 2011).

In earlier trials of AAV-mediated hFIX delivery in the treatment of haemophilia B, eight men were injected via the hepatic artery with AAV2-hFIX16 at doses ranging from 8 x 10^{10} vg/kg to 2 x 10^{12} vg/kg, during a period from 2001 to 2004. Long-term follow-up has revealed no late, vector-related complications (Manno *et al*., 2006; Wellman *et al*., 2012). In a subsequent trial conducted from 2009 to 2012, ten men were peripheral intravenous doses of a self-complementary AAV8-hFIX (AAV8-LP1-hFIXco) ranging from 2 x 10^{11} vg/kg to 2 x 10^{12} vg/kg, again with no late complications noted to-date (Nathwani *et al.,* 2011a; Davidoff *et al*., 2012). AAV5-hFVIII-SQ has been studied in an ongoing phase 1/2, dose-escalation study, with a total of 15 males receiving a peripheral intravenous dose ranging from 6 x 10^{12} vg/kg to 6 x 10^{13} vg/kg. Similar to the experience from prior AAV-mediated gene transfer studies, no late, vector-related complications have been observed to-date (Pasi *et al.*, 2017).

Potential immune response

Administration of AAV5-hFVIII-SQ leads to the production of a vector capsid specific antibody response. In addition, antibody responses specific for the transgene product, hFVIII-SQ, remain a possibility and is closely monitored in the clinic in the ongoing phase 1/2 study. In clinical trial results to date, anti-AAV5 vector capsid-specific antibodies have been detected after vector infusion, and the presence of these antibodies may inhibit vector transduction upon administration of subsequent doses of AAV5 vectors and may preclude patients from further treatment with AAV vectors.

There is a possibility that subjects infused with AAV5-hFVIII-SQ could develop neutralising antibodies (inhibitors) to FVIII. Patients enrolling in the clinical study are required to have a minimum of 150 exposure days to recombinant FVIII without development of an inhibitor response. To date, no patients in the ongoing phase 1/2 study have tested positive in the Nijmegen modified Bethesda assay for inhibitors.

A cell-mediated response to AAV5-hFVIII-SQ could give rise to elevated transaminases. In a previous study of AAV-mediated gene therapy for haemophilia B, two patients infused with either 4 x 10^{11} vg/kg or 2 x 10^{12} vg/kg of AAV2-hFIX vector developed asymptomatic elevation of transaminases beginning four weeks after vector infusion and resolving to baseline by twelve weeks post vector infusion without specific treatment (Manno *et al.,* 2006). In addition, two of six subjects infused with 2 x 10^{12} vg/kg AAV8-hFIX vector developed elevation of hepatic transaminases. These subjects were treated with a short, tapering course of prednisolone and resolved the elevated transaminases; one suffered a modest reduction in FIX levels, but remained with a level greater than his baseline, while the other maintained his FIX level throughout the reported period of observation (Nathwani *et al.,* 2011a). Consistent with these observations, an increase in ALT has been observed in the majority of subjects administered BMN 270 in Study 270-201. All adverse events of ALT increased were reported as non-serious and Grade 1 in severity, and resolved to below the upper limit of normal. Patients with elevated ALT were treated with a tapering course of steroids either prophylactically or on demand.

Potential for insertional mutagenesis

AAV vectors have the potential to integrate into the genome of transduced cells. The major potential risk resulting from vector DNA integration into the host cell DNA is a risk of malignant transformation leading to cancer.

It is assumed that the greatest potential for integration would be into cells within the liver, but given the results of tissue distribution studies of NHPs with similar (AAV8 pseudotyped) vectors (AAV8-LP1-hFIXco; Nathwani *et al*., 2011b) the potential for integration into cells of other tissues also exists.

At high multiplicity of infection, wild type AAV integrates into human chromosome 19 in ~60% of latently infected cell lines. However, it has been demonstrated that only approximately 1 out of 1000 infectious units can integrate (Tenenbaum *et al*., 2003). The mechanism of this site-specific integration requires the AAV Rep proteins which are absent in AAV5-hFVIII-SQ. Accordingly, recombinant AAV (rAAV) do not integrate site-specifically. Random integration of vector sequences has been demonstrated in established cell-lines but only in some cases and at low frequency in primary cultures and *in vivo*. In contrast, Duan *et al.*, 1998 demonstrated prolonged persistence of head-to-tail circular intermediates in muscle tissue at 80 days post-infection, suggesting that a large percentage of rAAV genomes may remain episomal. It has also been shown that, following liver transduction, (Miao *et al.,* 1998; Miao *et al.,* 2000) rAAV is stabilized predominantly in a non-integrated form; however, integration does occur at some low level.

The site(s) of integration have been analysed using 454 pyrosequencing and bioinformatics analysis to characterize > 1000 integration sites from mouse liver injected at high doses with an AAV2-hFVIII vector. These data (Li *et al*., 2011) confirmed earlier reports (Nakai *et al*., 2005) of preferential integration into actively transcribed genes, CPG islands, and GC-rich regions. These data are relevant because both AAV2 and AAV5 vectors utilize the AAV2 ITRs, the vector element involved in integration. Integration of AAV5-hFVIII-SQ genome has not yet been evaluated experimentally, although based on a thorough literature review of similar vectors with AAV2 ITRs, the frequency of integration as measured for AAV vectors in mice, rabbits, nonhuman primates and humans is very low, at least several orders of magnitude below the spontaneous rate of mutation in human cells (Cole and Skopek, 1994).

It is important to note that the integration mechanism of AAV differs from that of other viruses, such as retroviruses. Retroviral vectors contain the proteins needed to cause double-stranded DNA breaks for integration. AAV vectors do not contain such proteins and must rely on the host's cellular machinery. In fact, it is currently thought that AAV vectors may preferentially integrate into genomic DNA regions that are already broken (Miller *et al.,* 2004).

A mouse model of AAV infusion into liver during the neonatal period led to an increased number of hemangiosarcomas and hepatocellular carcinomas (HCC) after prolonged periods, and for at least some of the HCC tumors, an integration event in mouse chromosome 12 was associated with the tumor tissue but not the normal adjacent tissue (Donsante *et al*., 2007). Other studies have not confirmed this association, although the studies may have been hampered by inadequate numbers of mice (Li *et al.,* 2011). Still, the risk of such an event is suspected to be low based on absence of malignant transformation in large haemophilic animals (haemophilia B dogs) followed for over ten years after AAV vector infusion (Niemeyer *et al.,* 2009). In addition, liver

ultrasounds carried out in 4/7 human subjects injected with AAV2-hFIX vector from 2001-2004 have shown no evidence of liver tumors. Of the remaining 3 subjects: 1 has died of causes unrelated to gene transfer, and autopsy showed no evidence of tumor in the liver; 1 was lost to follow-up; and 1 has declined to undergo a liver ultrasound but remains in good health (Wellman *et al.,* 2012).

Potential for germline transmission

In an AAV2-FIX vector liver gene transfer trial (Manno *et al.,* 2006), vector genome sequences were detected in semen; semen samples from all eight subjects cleared within sixteen weeks. A series of non-clinical studies was performed to demonstrate: 1) the transient nature of this finding in both humans and animals; and 2) that vector sequences were not detected in spermatocytes (Arruda *et al.,* 2001; Couto and Pierce, 2003; Couto *et al.,* 2004; Manno *et al.,* 2006). The sponsor of that study performed a serie of studies using rabbits, a valuable model for assessing germline transmission risk for humans. It was determined that following intravenous injection of AAV2, the duration of detection of vector genomes in the semen is dose-dependent and time-dependent, with genomes diminishing over time until they are completely undetectable; AAV infectious particles were only present up to day 4 post-injection and undetectable thereafter (Schuettrumpf *et al.,* 2006). The sensitivity of the nested PCR used in these studies was greater than 1 copy per 6000 cells. Rabbit studies were also performed with AAV8 vectors (Favaro *et al.,* 2009). In these studies, AAV2 and AAV8 vectors were compared, showing that the kinetics of vector clearance from semen was dose- and time-dependent but serotype-independent. Furthermore, AAV2 or AAV8 sequences were detected in the semen of vasectomized animals that lack germ cells, leading to the conclusion that the genitourinary tract, as well as the testis, contributes significantly to vector shedding in the semen. These studies are supported by findings in humans (Nathwani *et al.,* 2011a; see Section II.C.2.(i) 3), in which vector genomes were detected in semen only briefly after AAV8-LP1-hFIXco vector systemic administration; clearance was reported by 15 days post-administration. These results suggest that the risk of inadvertent germline transmission in males by AAV vectors is low.

(2) Comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;

AAV5 is not pathogenic and AAV5/FVIII is not expected to be, based on its lack of viral genome (*rep* and *cap* genes are deleted and replaced with hFVIII-SQ), subsequent inability to replicate and the benign nature of the expressed human transgene hFVIII. The transgene is fully human, nontoxic and not oncogenic.

Even though the FVIII gene is a human gene, there is only a limited homology to the endogenous gene since the FVIII coding sequence in the vector has been codon-optimized. The silent mutations at the nucleotide level will likely decrease the risk of insertional mutagenesis. Moreover, the target cells (hepatic) do not replicate at appreciable levels, and any theoretical risk of recombination between the vector and the endogenous FVIII gene would be limited to dividing cells due to accepted mechanisms of homologous recombination during DNA replication. Thus, even without codon optimization, the actual risk of vector insertion into a gene of any cell is several orders of magnitude below the risk resulting from the spontaneous mutation rate in human genes.

(3) Capacity for colonisation;

AAV5-hFVIII-SQ is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

Homologous recombination between AAV5-hFVIII-SQ and a wild type AAV could occur if both were present in the same cell in the presence of a helper virus (triple infection). However, such recombination could only result in the exchange of the hFVIII expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

In the rare event that wild type AAV, supplying the requisite replication gene products, were to co-infect a hepatocyte, along with a helper DNA virus such as adenovirus or herpes simplex virus and AAV5-hFVIII-SQ (a triple co-infection), it is possible that vector replication could occur. The likelihood of such an occurrence is extremely low, and the resulting virologic outcome would be synthesis of B and wild type AAV, both intrinsically non-pathogenic viruses.

The capacity for colonisation is also affected by the level and route of shedding of the GMO from patients following administration.

BMN 270 Vector Shedding in Study 270-201; Ongoing phase 1/2 study

Vector shedding is being studied in all studies; blood, saliva, feces, semen, and urine are tested to monitor shedding through bodily fluids. Results to date are available from 270 201, as published in (Rangarajan et al. 2017 NEJM).

Vector shedding is monitored using a very sensitive qPCR-based method. Clearance in a fluid compartment from each patient is defined as having 3 consecutive negative samples.

Vector DNA was detected by qPCR in blood, semen, saliva, urine, and feces from all 15 subjects in 270-201, with peak levels within 4 weeks after BMN 270 infusion. Overall, all biofluids show decreasing quantities of residual vector DNA over the course of the study. In the high dose cohort, the fastest clearing biofluid was urine; all subjects had cleared urine by Week 28 (range Weeks 6-28). In semen, 4 of 7 subjects cleared vector DNA by Week 36 (range Weeks 16-36). One subject cleared at week 56 and the other two had levels below the limit of detection at week 52. A purified sperm sample alongside the whole semen sample was collected from these two participants and the result of PCR analysis for the presence of BMN 270 DNA sequence in sperm was negative. The entire high-dose cohort had cleared the saliva fluid compartment (range Weeks 40-52). No high-dose subjects cleared vector DNA in the feces by Week 52, but levels were below the limit of quantification.

(4) If the organism is pathogenic to humans who are immunocompetent:
Diseases caused and mechanism of pathogenicity including invasiveness and virulence, communicability, infective dose, host range, possibility of alteration, possibility of survival outside of human host, presence of vectors or means of dissemination, biological stability, antibiotic resistance patterns, allergenicity, availability of appropriate therapies.

Neither wild type AAV nor the experimental vector AAV5-hFVIII-SQ is known to be pathogenic to humans.

(5) Other product hazards.
None known or anticipated.

III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT

A. INFORMATION ON THE RELEASE

1. DESCRIPTION OF THE PROPOSED DELIBERATE RELEASE, INCLUDING THE PURPOSE(S) AND FORESEEN PRODUCTS

The release of the GMO will be made in the context of the clinical trials with protocol numbers 270-301 and 270-302. Both studies are Phase 3, single-arm, open-label studies designed to assess whether, in an expanded sample, BMN 270 can safely alter the clinical phenotype of hemophilia A patients with residual FVIII activity ≤ 1 IU/dLpreviously treated with FVIII prophylaxis. 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, bleed rate). As AAV-based treatment always leads to anti-capsid immune response, no true control group (for example with an empty AAV) is possible.

There will be approximately 60 sites globally participating in these studies, with 3 planned sites in Belgium. All Belgian sites will act as dosing site, where subjects from Belgium will be administered BMN 270.

2. FORESEEN DATES OF THE RELEASE AND TIME PLANNING OF THE EXPERIMENT INCLUDING FREQUENCY AND DURATION OF RELEASES

The start date for the study is planned for May 2019 in Belgium following approval of the Federal Agency for Medicines and Health Products and positive opinion from the Ethics Committee.
Up to 130 patients (Study 270-301) and 40 patients (Study 270-302) may be dosed globally. Participants will be observed at the infusion centre for any immediate toxicity of the procedure. Regular follow-up visits after infusion of BMN 270 will be performed according to the protocol

3. PREPARATION OF THE SITE PREVIOUS TO THE RELEASE

All Belgian sites will act as dosing site, where subjects from Belgium will be administered

BMN 270. The sites will be trained on drug assignment, receipt, dispensing, storage, and accountability procedures.

All involved personnel on the sites will be trained in best biosafety practices to be applied during preparation in the pharmacy, transport to the administration room, precautions during administration and disposal of any biological waste. Such training will involve, among others, wearing adapted protective clothing and gloves and the surface decontamination.

In addition to receiving a site initiation visit by the sponsor that reviews investigational product storage, handling, dilution and administration according to the Study Pharmacy manual, the dosing site will complete an in service training on use of the administration syringe

4. SIZE OF THE SITE

The study activities will be performed at the study sites. The sites have hospital pharmacy, exam rooms, infusion area, laboratory, biosamples collection and processing area, liver ultrasound and ECG areas, document storage spaces and access to all the facilities offering the maximum care level.

5. METHOD(S) TO BE USED FOR THE RELEASE

The release will be performed at each dosing facility by a slow intravenous infusion.

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.

BMN 270 will be infused intravenously to deliver the entire volume over an approximately 1- 2 hour period, depending on the dose. In any event, once the infusion begins, the entire volume of the prepared IP should be administered so that the subject receives the entire calculated Total Subject Dose of BMN 270 (as long as the PI deems it safe to do so). Patients will be observed at the hospital for any immediate toxicity of the procedure. Subjects will remain at the clinic for at least 8 hours to observe for any immediate toxicity of the procedure in patient observation can be extended beyond 8 hours if needed per Investigator discretion or transfer to an inpatient facility for hospitalization may occurbased on the evaluation and judgment of the Clinical Principal Investigator after consultation with the Medical Monitor.

Regular follow-up visits after infusion of BMN 270 and the 8h observation period will be done according to the protocol.

6. QUANTITIES OF GMOS TO BE RELEASED

Subjects will not be randomized, and will receive one dose of BMN 270. A maximum of 7 patients will be treated in Belgium in Study 270-301, and up to 3 patients will be treated in Study 270-302, each receiving a single dose of BMN 270 either at a dose of 6E13 vg/kg (Study 270-301) or 4E13 vg/kg (Study 270-302).The solution will be injected at 2E13 vg/ml as a pure solution. Therefore, the maximum quantity of investigational product to be utilized is 3.36E16 vg in Study 270-301 and 0.96E16 vg in Study 270-302, assuming an average subject weight of 80 kg.

The vector is replication incompetent, even in the presence of a helper virus, so release of the vector following administration is limited to environmental release of the vector by subject shedding during a limited time period following administration.

7. DISTURBANCE ON THE SITE (TYPE AND METHOD OF CULTIVATION, MINING, IRRIGATION, OR OTHER ACTIVITIES)

There will be no disturbance at the sites of dose preparation/administration.

8. WORKER PROTECTION MEASURES TAKEN DURING THE RELEASE

The investigational product will be provided to all Belgian sites on a subject-by-subject basis, following confirmation of subject eligibility and a review of registration documents/essential documents.

Within the sites, the investigational product will be stored in a secure environment. It will be stored in a freezer located in a closed room with restricted access. Inside the freezer, the product will be kept inside a closed box. The box can only be opened by personnel who has been included in the study and will be identified with the study code and separated from the other medication.

Patients will be placed into an isolated hematology ward room at the time of the investigational product administration at the sites.

A qualified investigator with specific training on the protocol will be responsible for gene transfer material receipt from the Sponsor, storage, documentation of traceability of product at the investigational site, preparation (dilution and combination of components) on the day of administration and disposal.

In addition, all study personnel will be trained on the Clinical Trial Protocol and Handling Instructions as part of the study site initiation.

As a minimum, institutional risk assessments are required to determine appropriate precautions for the work tasks and processes.

AAV5-hFVIII-SQ vector is derived from a virus and should be considered and handled as an infectious agent. Given the non-replicative nature of the modified organism, the nature of the transgene, and the fact that the parent organism is not known to be pathogenic, BSL-1 procedures are appropriate in the clinical setting.

Although level 1 standard practices are enough, the standard practices used correspond to BSL2 level:

- Restricted access
- Secure storage
- Training of personnel
- Biosafety cabinet for dose preparation.
- Availability of Personal Protective Equipment (gowns, gloves, caps, masks and goggles)
- Established routine practices for dealing with potentially biohazardous materials such as patient samples/fluids and medical waste (autoclaves, sharps bins, incinerators, disinfectants and appropriate cleanable surfaces).

The worker protection measures proposed for the preparation and administration of AAV5-hFVIII-SQ are therefore in line with those recommended globally for the handling of BSL-1/2 organisms in a research setting. Good Microbiology Techniques will be applied as a minimum for handling the GMO: the use of gloves and lab coat or scrub suit during the manipulation, washing one's hands after manipulation, disposing of the material used as biological waste, disinfecting the area after the manipulation, applying sharp objects handling policies, having collection and disinfection of waste procedures and

materials and an action plan in case of an accident.

9. POST-RELEASE TREATMENT OF THE SITE

All disposable materials used in the infusion rooms or preparation laboratories during preparation or administration of investigational product (including but not limited to gloves, masks, syringes, needles, catheter and tubing) that come into contact with the product shall be disposed of as biohazardous materials according as biohazard waste.

- All disposable materials will be disposed of in approved containers for biological residues depending on the amount of material produced.
- Injectable or sharp materials (syringes-needles, broken glass, etc.) will also be disposed in specific approved containers for injectable material with appropriate volume to the type of material to be discarded.
- These containers will be closed hermetically once full. The containers will be filled up to 75% of their capacity.
- Both types of containers will be collected by hospital's personnel designated for these tasks and transported to the final waste warehouse.
- The waste will be decontaminated either by incineration or autoclavation by saturated steam autoclave using validated fractional vacuum cycles by an authorized hazardous waste management company.
- The transfer of the containers to hazardous waste management company will be done by an authorized companies.

This waste management procedure will also apply to the unused investigational product and vials, stopper and crimp seal materials as well as for the materials used for the collection of samples. Non-disposable materials, equipment and surfaces will be decontaminated will be decontaminated with broad-spectrum disinfectants with proven activity against non-enveloped viruses. Solutions such as Surfa'safe Spray (didecyldimethylammonium chloride) or Umonium (benzalkonium chloride, isopropilic alcohol and lauromyristic alcohol) may be used.

Any spillage produced will be collected following the procedure indicated in section V.D.2 and all surfaces will be decontaminated with the disinfectants indicated above.

The instructions and documentation for the destruction of the IMP and any waste generated will be followed and documented by a trained investigator at the study site.

10. TECHNIQUES FORESEEN FOR ELIMINATION OR INACTIVATION OF THE GMOS AT THE END OF THE EXPERIMENT

Instructions for, and worksheets documenting (contained in the Study Pharmacy Manual) the destruction of unused undiluted and diluted Investigational product, along with associated generated waste will be followed and documented by the qualified and trained investigator at the sites.

In general, the GMO will be eliminated by disposing it of as biological waste and its subsequent sterilization by incineration or in a saturated steam autoclave. Any contaminated material or surface will be disinfected with broad-spectrum disinfecting solutions with specific action against non-enveloped viruses, as Surfa'safe Spray (didecyldimethylammonium chloride) or Umonium (benzalkonium chloride, isopropilic alcohol and lauromyristic alcohol).

The vector cannot be replicated, even in the presence of a helper virus, however, it can be present in the patient's excretions for a limited period of time after administration. For this reason, patients included in the study will be trained in the treatment of excretions that may contain the GMO, especially urine and feces. For this purpose, at least half a cup of sodium hypochloride (desinfectant bleach) will be added after each urine or stool by dropping it down the walls of the toilet before dumping it. Patients will wash their hands with soap after using the bathroom. This treatment will be continued until the vector's presence controls recommend so (3 negative consecutive samples). Similarly, male patients will refrain from sexual intercourse due to the potential presence of the GMO in the semen.

11. INFORMATION ON, AND RESULTS OF, PREVIOUS RELEASES OF THE GMOS, ESPECIALLY AT DIFFERENT SCALES AND IN DIFFERENT ECOSYSTEMS

Viral vectors, including vectors derived from adeno-associated virus (AAV), are frequently used in gene therapy. The consequences of the release in the environment are not entirely known yet, however some data are available in the literature.

In preclinical studies in animals the AAV5 capsid has behaved similarly to AAV8 fordelivery to liver (Nathwani, 2011, Mol.Ther.), (Davidoff, 2005, Mol.Ther.), (Nathwani,

2007, Blood). The AAV5 biodistribution after intravenous delivery has been well studied in several animal species, including mice, rabbits and nonhuman primates, and primarily targets liver with some extra hepatocyte spread (Davidoff, 2005, Mol.Ther.), (Paneda, 2009, Hum.Gene Ther.), (Favaro, 2011, Hum.Gene Ther.), (Gao, 2006, Mol.Ther.), (Paneda, 2013, Hum.Gene Ther.)

Table 4: AAV5 Vector Biodistribution

Biodistribution of AAV5 vectors after intravenous dosing [a]					
Species	Mouse	Rabbit	Monkey (Cyno)	Monkey (Rhseus)	Monkey (Rhesus)
Dose (vg/kg)	5×10^{12}	1×10^{13}	1×10^{13}	5×10^{13}	4×10^{12}
Time (days)	92	210	436	30	180
Reference	Paneda et al 2009 (35)	Favoro et al 2011 (36)	Gao et al 2006 (37)	Paneda et al 2013 (38)	Davidoff et al 2005 (33)
Organ	Amount of Vector genomes relative to liver 1				
Liver	1.0	1.0	1.0	1.0	1.0
Spleen	<0.002	0.4	0.01	1.0	
Heart	<0.002	0.8	0.01	< 0.002	
Lung	<0.002	0.6	0.01	< 0.002	
Kidney	<0.002	0.1	0.004	< 0.002	0.12
Gonad	-	-	0.0001	-	
Testis	<0.002	0.1	-	< 0.002	0.01
Ovary	<0.002	Nd[b]	-	< 0.002	
Uterus	<0.002	Nd	-	Nd	
Small Bowel	Nd	Nd	0.001	Nd	
Colon	Nd	Nd	0.001	Nd	
Bone Marrow	Nd	Nd	0.0001	Nd	
Lymph Node	Nd	Nd	Nd	< 0.002	
Bladder	Nd	0.4	Nd	Nd	
Adrenal	Nd	Nd	Nd	1.0	
Prostate	Nd	0.05	Nd	Nd	
Semen	Nd	0 (at 30 days)	Nd	Nd	

a The original published data are reported using various units including vector genomes per diploid cell content or vector genomes per µg of cell DNA. In this table, for each study, all data have been normalized relative to the value for liver.
b Nd = not done

The 2007 joint EMEA and ICH workshop on viral/vector shedding determined that while rAAV is extensively biodistributed and shedding is known, the virus is nonpathogenic and risks are estimated to be very low (EMEA/ICH Workshop 2007) In studies of AAV2 vectors in cystic fibrosis (Moss *et al*, 2004, Chest) and HIV vaccines (Vardas *et al*., 2010,.AIDS Res Hum Retroviruses) administered via aerosol or intramuscularly, respectively, at doses as high as 1×10^{13} vector genomes, most samples were negative and those that were positive were at less than 1/1,000,000 of the dose administered even at 2 hours after dosing. Stool and urine samples were negative for all samples. In studies of AAV vectors for haemophilia B patients that were administered doses as high as 1×10^{14} vector genomes, persistence in saliva, urine and semen was 1 week or less. (Manno *et a*l., 2003, Blood) Since rAAV is non-replicating, even in the presence of helper virus, there is no reason to believe that the added DNA will spread from the human subject to other

persons or to the environment.

B. INFORMATION ON THE ENVIRONMENT (BOTH ON THE SITE AND IN THE WIDER ENVIRONMENT):

1. GEOGRAPHICAL LOCATION AND GRID REFERENCE OF THE SITE(S) (IN CASE OF NOTIFICATIONS UNDER PART C THE SITE(S) OF RELEASE WILL BE THE FORESEEN AREAS OF USE OF THE PRODUCT)

The IMP dosing will take place at the following study sites in Belgium:

- Universitair Ziekenhuis Leuven, Herestraat 49, 3000 Leuven
- Cliniques Universitaires Saint-Luc, Avenue Hippocrate,10 1200 Bruxelles
- Universitair Ziekenhuis Antwerpen, Wilrijkstraat 10, 2650 Edegem

2. PHYSICAL OR BIOLOGICAL PROXIMITY TO HUMANS AND OTHER SIGNIFICANT BIOTA

Investigational product administration will be performed at each dosing facility, Subjects will be monitored either in a recovery area or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

Given the nature of the product administration (intravenous) and the transient/low levels of shedding expected, the risk of unintended exposure to BMN 270 to humans and other biota is minimal. Nearly all of the low level of vector genomes that are present in body fluids appear to be cell associated, and not present as free vector particles. This makes it even less likely that there could be horizontal transmission of infectious genomes to others. Also, none of the vector genomes are detected in sperm so there is no likelihood of vertical transmission. Furthermore, on the basis of the vector construct, it is considered that AAV5-hFVIII-SQ vector construct has been appropriately engineered with characteristics such that the adeno-associated viral vector has been sufficiently attenuated resulting in disablement of the vectors ability to infect, replicate or survive outside of humans.

3. PROXIMITY TO SIGNIFICANT BIOTOPES, PROTECTED AREAS, OR DRINKING WATER SUPPLIES

Given the nature of the product administration, scale of release and procedures for waste treatment, the exposure to significant biotopes, protected areas and drinking water supplies is expected to be negligible.

4. CLIMATIC CHARACTERISTICS OF THE REGION(S) LIKELY TO BE AFFECTED

The clinical trial of AAV5-hFVIII-SQ will occur in Belgium which has a temperate climate. The risk of release of AAV5-hFVIII-SQ in to the environment is unrelated to climatic characteristics.

The stability of AAV5-hFVIII-SQ in the environment is unchanged from that of wild-type AAV.

The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in an infusion room located at the dosing site. Subjects will recover either in a recovery area of the infusion room or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

5. GEOGRAPHICAL, GEOLOGICAL AND PEDOLOGICAL CHARACTERISTICS

The risk of release of AAV5-hFVIII-SQ into the environment is unrelated to these characteristics. The stability of AAV5-hFVIII-SQ in the environment is unchanged from that of wild-type AAV.

The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in an infusion room at the dosing site. Subjects will recover either in a recovery area off the infusion room or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

Outside of the dosing sites, following discharge of subjects home where some shedding may occur, given the nature of the product administration (intravenous) and the transient/low levels of shedding expected of a replication incompetent GMO, the risk of unintended exposure to AAV5-hFVIII-SQ to humans and other biota is minimal.

6. FLORA AND FAUNA, INCLUDING CROPS, LIVESTOCK AND MIGRATORY SPECIES

Given the nature of the product administration (intravenous) and the transient/ low levels of shedding expected, the risk of unintended exposure of flora and fauna to AAV5-hFVIII-SQ is minimal.

The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed at each dosing facility Subjects will recover within the dedicated dosing facility for minimum of 8 hours post infusion. All clinical site staff both at the dosing facility as well as at the primary research site will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

7. DESCRIPTION OF TARGET AND NON-TARGET ECOSYSTEMS LIKELY TO BE AFFECTED

No ecosystems are targeted in the use of AAV5-hFVIII-SQ. Ecosystems are not expected to be affected.

8. A COMPARISON OF THE NATURAL HABITAT OF THE RECIPIENT ORGANISM WITH THE PROPOSED SITE(S) OF RELEASE

The natural habitat of the recipient organism is the human host. The release will be in a hospital setting as part of a clinical study. However some low level shedding may occur once subjects are discharged from the Hospital following dosing.

9. ANY KNOWN PLANNED DEVELOPMENTS OR CHANGES IN LAND USE IN THE REGION WHICH COULD INFLUENCE THE ENVIRONMENTAL IMPACT OF THE RELEASE

None known.

IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOS AND THE ENVIRONMENT

A. CHARACTERISTICS AFFECTING SURVIVAL, MULTIPLICATION AND DISSEMINATION

1. BIOLOGICAL FEATURES WHICH AFFECT SURVIVAL, MULTIPLICATION AND DISPERSAL

AAV5-hFVIII-SQ is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV *in vitro*, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987). It is not known whether zoonosis occurs in nature, nor whether other species can act as carriers or vectors under natural conditions

The survival of AAV5-hFVIII-SQ outside of the host is expected to be the same as wild- type AAV.

2. KNOWN OR PREDICTED ENVIRONMENTAL CONDITIONS WHICH MAY AFFECT SURVIVAL, MULTIPLICATION AND DISSEMINATION (WIND, WATER, SOIL, TEMPERATURE, PH, ETC.)

Environmental conditions which may affect survival of AAV5-hFVIII-SQ outside the host are temperature, pH and environmental humidity. AAV5-hFVIII-SQ is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

3. SENSITIVITY TO SPECIFIC AGENTS

The genetic modifications made during the construction of AAV5-hFVIII-SQ from wild type AAV5 are not expected to affect its sensitivity to physical and chemical inactivation.

BMN 270 is destroyed by Alcohol 70° or Bleach solution, and cleaning agents or hand-sanitizers that are effective against non-enveloped viruses. In addition, it can be destroyed by high pH, UV light, ionizing radiation, or by incineration.

B. INTERACTIONS WITH THE ENVIRONMENT

1. PREDICTED HABITAT OF THE GMOS

The predicted habitat of AAV5-hFVIII-SQ is humans where it may persist in a latent state. AAV5-hFVIII-SQ is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

2. STUDIES OF THE BEHAVIOUR AND CHARACTERISTICS OF THE GMOS AND THEIR ECOLOGICAL IMPACT CARRIED OUT IN SIMULATED NATURAL ENVIRONMENTS, SUCH AS MICROCOSMS, GROWTH ROOMS, GREENHOUSES

AAV5-hFVIII-SQ is a replication-incompetent virus derived from AAV5. The genetic modifications do not affect its natural host and tissue tropism.

No specific studies have been conducted regarding transmission of AAV5-hFVIII-SQ between humans or animals.

No shedding studies have been performed in animals, shedding will be monitored as part of the clinical trial.

3. GENETIC TRANSFER CAPABILITY

(a) post-release transfer of genetic material from GMOs into organisms in affected ecosystems;

BMN 270 is incapable of replicating itself which may be considered a potential safety benefit, when compared to integrating vectors with the ability to replicate, in that the total dose of virus administered to a patient can be carefully controlled and there is minimal risk of unintended transmission.

(b) post-release transfer of genetic material from indigenous organisms to the GMOs;

AAV5-hFVIII-SQ is a replication-incompetent virus derived from AAV5. The genetic modifications do not affect its natural host and tissue tropism. No transfer of genetic material between the GMO and other organisms is predicted.

The transfer of genetic material is therefore limited to the theoretical genetic exchange of DNA by homologous recombination with wild type AAV which could only occur if human cells were simultaneously infected with both wild type AAV and BMN AAV5-hFVIII-SQ, in the presence of a helper virus. In the case of AAV5-hFVIII-SQ, such recombination could only result in the exchange of the hFVIII expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV5-hFVIII-SQ (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, and would only result in the production of more wild type AAV and more AAV5-hFVIII-SQ vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining).

4. LIKELIHOOD OF POSTRELEASE SELECTION LEADING TO THE EXPRESSION OF UNEXPECTED AND/OR UNDESIRABLE TRAITS IN THE MODIFIED ORGANISM

The selective pressure on AAV5-hFVIII-SQ will be towards reversion to wild-type, since both gene deletions (*rep* and *cap*) are required for rescue and replication of the organism in its host species.

The likelihood of this reversion is considered low, since it would require genetic exchange by homologous recombination with wild-type AAV which could only occur if human cells were simultaneously infected with both wild type AAV, AAV5-hFVIII-SQ and a helper virus (e.g. adenovirus).

The transgene (human coagulation Factor VIII) is not expected to confer any advantage to the GMO in terms of survival and selective pressure.

5. MEASURES EMPLOYED TO ENSURE AND TO VERIFY GENETIC STABILITY. DESCRIPTION OF GENETIC TRAITS WHICH MAY PREVENT OR MINIMISE DISPERSAL OF GENETIC MATERIAL. METHODS TO VERIFY GENETIC STABILITY

AAV5-hFVIII-SQ is stable by design in that the vector DNA is approximately the same size as the viral genome and it contains no viral genes. The investigational product‘s stability is assured by a well-characterized manufacturing process, in-process testing and batch release testing.

6. ROUTES OF BIOLOGICAL DISPERSAL. KNOWN OR POTENTIAL MODES OF INTERACTION WITH THE DISSEMINATING AGENT, INCLUDING INHALATION, INGESTION, SURFACE CONTACT, BURROWING, ETC.

There will be a single intravenous infusion of each study subject in the hospital setting of each dosing facility,

Other routes of exposure may occur by inhalation, contact with mucus membranes (eyes, nose and mouth), faecal-oral transmission and occasionally waterborne transmission. The parent AAV virus is disseminated primarily by contact of mucus membranes. Direct contact with surfaces, exposure to aerosols and abrasions (sharps) may facilitate transmission.

An accidental spill of the investigational product at pharmacy or in the infusion room at the dosing sites, or by shedding of vector from subjects could lead to environmental contamination theoretically resulting in unintended transfer to humans or animals. wtAAV5 infects humans and primates, but no other known environmental organisms, and the vector would be expected to behave similarly.

7. DESCRIPTION OF ECOSYSTEMS TO WHICH THE GMOS COULD BE DISSEMINATED

Not applicable. BMN 270 is “gutless” and cannot replicate under any conditions.

8. POTENTIAL FOR EXCESSIVE POPULATION INCREASE IN THE ENVIRONMENT

Not applicable. BMN 270 is “gutless” and cannot replicate under any conditions

9. COMPETITIVE ADVANTAGE OF THE GMOS IN RELATION TO THE

UNMODIFIED RECIPIENT OR PARENTAL ORGANISM(S)

Because the most of the viral genome is absent and there are no viral genes the GMO is therefore at a competitive disadvantage when compared to its parent strain / wild type AAV. The transgene (human coagulation Factor VIII) is not expected to confer any advantage to the GMO in terms of survival and selective pressure.

10. IDENTIFICATION AND DESCRIPTION OF THE TARGET ORGANISMS IF APPLICABLE

The target organism is humans, specifically eligible, consenting adult males with X- linked severe Haemophilia A.

11. ANTICIPATED MECHANISM AND RESULT OF INTERACTION BETWEEN THE RELEASED GMOS AND THE TARGET ORGANISM(S) IF APPLICABLE

Normal Factor VIII is synthesized and processed in endothelial cells, which line blood vessels in the liver. In Haemophilia A, an insufficient amount of FVIII is made, or the factor produced is deficient in its function. BMN 270 is a vector coding for a functional form of FVIII. The vector is brought into hepatocytes via binding to viral capsid receptors on the surface of liver cells, then the capsid proteins are removed, and the DNA translocates to the nucleus, where it remains in a stable episomal form. In the nucleus, the transgene codes for the FVIII protein that is secreted into the circulation.

Activated factor VIII (FVIII) is the cofactor for activated factor IX, which is necessary to promote effective clot formation. A low concentration of FVIII is sufficient to prevent spontaneous bleeding from occurring.

Haemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males (Nathwani, 1992, Baillieres Clin. Haematol). The clinical phenotype of haemophilia A patients, who have quantitatively deficient activity levels, is largely governed by the level of residual FVIII 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. Treatment of severe HA in the developed world consists of intravenous injection of plasma derived or recombinant FVIII protein (rhFVIII) concentrates both as prophylaxis at least 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The half-life for rhFVIII 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 a debilitating multiple-joint arthropathy, and substantially increased risk of death from haemorrhage throughout life (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 (Mahlangu, 2014, Blood). However, these longer acting FVIIIs remain dependent on multiple but fewer infusions to maintain critical levels of FVIII activity in severe HA patients.

Gene therapy, in contrast, offers the potential of disease-modifying therapy by continuous endogenous production of active hFVIII following a single intravenous administration of vector. 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 small amounts (200 ng/ml) in the plasma. Modest increases in the level of hFVIII (>1% of normal activity results in increased expression of >2 ng/ml protein) can ameliorate the severe form of the disease. Thus, relatively small changes in endogenous hFVIII activity results in clinically relevant improvements in disease phenotype. Finally, the response to gene transduction can be assessed using simple quantitative rather than qualitative endpoints that are easily assayed in most clinical laboratories.

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 safety profile and can direct long-term transgene expression from post-mitotic tissues such as the liver (Nathwani, 2005, Curr.Hematol Rep.). 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 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 bleedings. Thus, gene therapy treatment has resulted in a substantial improvement in their quality of life (Nathwani, 2011, Mol.Ther.). Similar encouraging results have emerged from clinical trials of AAV mediated gene transfer to the retina for the treatment of Leber's congenital amaurosis (Bainbridge, 2008, NEJM; Maguire, 2009, Lancet; Simonelli, 2010, Mol.Ther.).

BMN 270 is an AAV5-based gene therapy vector that expresses an active form of rhFVIII under the control of a liver-selective promoter 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 an ongoing first-in-human study designed to assess the relationship of vector dose to the augmentation of residual FVIII activity to the levels that alter clinical phenotype.

12. IDENTIFICATION AND DESCRIPTION OF NON-TARGET ORGANISMS WHICH MAY BE ADVERSELY AFFECTED BY THE RELEASE OF THE GMO, AND THE ANTICIPATED MECHANISMS OF ANY IDENTIFIED ADVERSE INTERACTION

wtAAV5 is not known to infect any organisms in the environment except primates. There is a chance that gene transfer could be made to other humans, however because the amount would be so low and the GMO is replication incompetent (even in the presence of helper virus) the result would be negligible.

13. LIKELIHOOD OF POSTRELEASE SHIFTS IN BIOLOGICAL INTERACTIONS OR IN HOST RANGE

The likelihood of post-release shifts in biological interactions or host range is negligible.

AAV enters cells by interaction of specific viral capsid epitopes with cell surface receptors. The inserted gene in AAV5-hFVIII-SQ is hFVIII, a human clotting factor that is packaged in viral capsid proteins derived from AAV5, and therefore would not be expected to alter the host range or cell tropism of the virus. The gene deletions in AAV5-hFVIII-SQ prevent the ability of the virus to replicate independently, but do not affect the packaging viral capsid proteins so would not be expected to have any effect on host range or cell tropism.

14. KNOWN OR PREDICTED INTERACTIONS WITH NON-TARGET ORGANISMS IN THE ENVIRONMENT, INCLUDING COMPETITORS, PREYS, HOSTS, SYMBIONTS, PREDATORS, PARASITES AND PATHOGENS

None known or predicted.

15. KNOWN OR PREDICTED INVOLVEMENT IN BIOGEOCHEMICAL PROCESSES

None known or predicted.

16. OTHER POTENTIAL INTERACTIONS WITH THE ENVIRONMENT

None known or predicted.

V. INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

A. MONITORING TECHNIQUES

1. METHODS FOR TRACING THE GMOS, AND FOR MONITORING THEIR EFFECTS

1.1. Monitoring during treatment of patients

On the day of the BMN 270 infusion, vital signs will be monitored hourly for 8 hours in the infusion center and observed to monitor for adverse effects related to the procedure. Prior to discharging subjects from the clinic, the Investigator or designee should instruct subjects how to recognize signs and symptoms of potential (delayed) hypersensitivity reactions and anaphylaxis, and to contact a medical practitioner or seek emergency care in case of such an event.

Study investigators will monitor subjects throughout treatment and will report adverse effects according to the requirements stipulated in the protocol. Vector shedding will be monitored at several timepoints after administration using a very sensitive qPCR-based method.

Factor VIII activity/antigen will be monitored at each study visit.

Patients will be provided with the written instructions for semen and feces collection. Other samples such as blood and urine will be analyzed and collection instructions are available in the lab manual. These are not part of the patient tool as blood and urine will be collected on site and not at a patient's home.

1.2. Follow-up of patients after treatment

Patients will be subject to long term follow-up (5 years) post-dose.

Vector shedding will also be extensively studied in the present clinical trial.

Subjects who do not respond to BMN 270 treatment (ie, treatment failure may, at the Investigator's discretion and after discussion with the Medical Monitor, follow an abbreviated visit schedule after Week 52 of the study.
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 until vector shedding has cleared.

2. SPECIFICITY (TO IDENTIFY THE GMOS, AND TO DISTINGUISH THEM FROM THE DONOR, RECIPIENT OR, WHERE APPROPRIATE THE PARENTAL ORGANISMS), SENSITIVITY AND RELIABILITY OF THE MONITORING TECHNIQUES

The vector genome contains unique sequences that are not expected to be found in clinical samples not exposed to the vector. Polymerase chain reaction based methods using vector genome specific primers can be used to detect GMO genetic elements with high sensitivity (see Part II. Section A.5 and II.A.6).

3. TECHNIQUES FOR DETECTING TRANSFER OF THE DONATED GENETIC MATERIAL TO OTHER ORGANISMS

No plans for detecting transfer of genetic material to other organisms are considered necessary.

4. DURATION AND FREQUENCY OF THE MONITORING

Each subject's participation will last approximately 52 weeks for the active phase of the study and patients will be followed in total for 5 years)

B. CONTROL OF THE RELEASE

1. METHODS AND PROCEDURES TO AVOID AND/OR MINIMISE THE SPREAD OF THE GMOS BEYOND THE SITE OF RELEASE OR THE DESIGNATED AREA FOR USE

Preparation of the investigational product will take place in an approved hospital environment. The administration of the investigational product will be by authorized trained personnel at each dosing facility, according to good clinical practice and the study protocol. The primary mode of containment during the IV administration procedure is application of Standard/Universal Precautions for infectious materials. In the laboratory personnel performing the procedure will wear lab coat, gloves, protective googles and surgical masks. Labs for processing clinical samples, e.g. bloods etc. would use standard precautions for bodily fluids.

All personnel involved in the administration of investigational product must attend an in-service training on the proper method for administration and participate in a dry run of its setup and operation prior to infusing the first subject. The investigational sites abide by all EU, country and self-imposed guidelines regarding the conduct of clinical trials, as well as the appropriate biosafety regulations required by the EMA for gene therapy medicinal research. We believe that research conducted within this framework adequately mitigates the risks of such research to the public health and therefore no additional measures will be undertaken. Only qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment will undertake the preparation, handling and safe disposal of BMN 270.

Destruction of unused IP and destruction or decontamination of all materials that may have been contaminated by IP is discussed in the section on waste treatment.

2. METHODS AND PROCEDURES TO PROTECT THE SITE FROM INTRUSION BY UNAUTHORISED INDIVIDUALS

AAV5-hFVIII-SQ will be administered to the patient by a medical professional at each dosing facility.

The first batch of IMP will be delivered at study start and then stored until it is used for treatment, or it has to be destroyed/returned to the manufacturer for any reason. The longest possible storage time is therefore until expiry.

The product itself will be stored prior to administration in a locked - freezer which is located in an area with restricted access. Inside the freezer the IMP will be stored in a locked barred

box - this box can only be opened by study team members and is labelled with the study code, so that study medication is clearly labelled as such and separated from other medication.

At the dosing facilities the IMP will be transported safely from the secure IMP storage room to the pharmacy, where the infusion will be prepared, and from the pharmacy to the infusion room, where the IMP infusion will be administered by study personnel who has been trained The transport will done in a closed and hermetic internal biological transport container designated for this purpose (approved secondary container for transport of category B infectious substances may be used for this purpose). Container size will be adequate for the size, shape or quantity of the material to be transported.

Samples of study participants will be handled only by qualified study personnel in a closed and hermetic internal biological transport container designated for this purpose (approved secondary container for transport of category B infectious substances may be used for this purpose).. The samples will be placed inside the container immediately after they have been taken and transported to the local lab where they are processed as required and then packed for shipment. Samples of study participants will be stored at sites until shipment to the central laboratory. All samples will be shipped on the day of collection to the central laboratory in Switzerland. Access to study samples will be restricted to limited number of persons, i.e. study personnel and members of the laboratory department.

Intrusion by unauthorized individuals is therefore considered adequately controlled.

3. METHODS AND PROCEDURES TO PREVENT OTHER ORGANISMS FROM ENTERING THE SITE

No other procedures are considered necessary to prevent other organisms from entering the site, since AAV5-hFVIII-SQ is a replication-incompetent version of wild-type AAV5. Within a medical facility, general pest control and cleaning procedures will be in place as dictated by site specific procedures for general hygiene.

C. WASTE TREATMENT

1. TYPE OF WASTE GENERATED

Waste generated from the preparation and infusion of AAV5-hFVIII-SQ will be limited to:

- Used vials of the Investigational Medicinal Product
- Used preparation equipment: syringes, needles, vials
- Used infusion bags and infusion kits
- Bags used for in-house transportation of potentially contaminated equipment.
- Cleaning material and items used to clean injected area
- Personal Protective Equipment used during dose preparation and administration

-

2. EXPECTED AMOUNT OF WASTE

All sites in Belgium will involve manipulation of the therapy.

The expected amount of waste is as follows:

During the dilution needle(s) and syringe(s) will come into contact with the investigational product. During the catheterization procedure a high pressure tubing line and a catheter will contact the investigational product. The remaining waste generated will be consistent with a typical catheterization procedure resulting in the generation of a few biohazard waste bags (infectious clinical waste bags) of potentially contaminated materials (for example, disposable gloves, miscellaneous laboratory absorbent material, and other routine pharmacy and lab disposables).

Each administration will result in the waste identified above.

3. DESCRIPTION OF TREATMENT ENVISAGED

AAV5-hFVIII-SQ is a replication-deficient non-pathogenic virus which is considered to present a much lower hazard to human health than other human biological waste which is frequently disposed of in medical facilities.

AAV5-hFVIII-SQ is sensitive to inactivation by a variety of commonly available physical and chemical methods.

All disposable materials used in the infusion rooms or preparation laboratories during preparation or administration of investigational product (including but not limited to gloves, masks, syringes, needles, catheter and tubing) that come into contact with the product shall be disposed of as biohazardous materials according as biohazard waste:

- All disposable materials will be disposed of in approved containers for biological residues depending on the amount of material produced.
- Injectable or sharp materials (syringes-needles, broken glass, etc.) will also be disposed in specific approved containers for injectable material with appropriate volume to the type of material to be discarded.
- These containers will be closed hermetically once full. The containers will be filled up to 75% of their capacity.
- Both types of containers will be collected by hospital's personnel designated for these tasks and transported to the final waste warehouse.
- The waste will be decontaminated either by incineration or autoclavation by saturated steam autoclave using validated fractional vacuum cycles by an authorized hazardous waste management company.
- The transfer of the containers to hazardous waste management company will be done by an authorized companies.

This waste management procedure will also apply to the unused investigational product and vials, stopper and crimp seal materials as well as for the materials used for the collection of samples until it is indicated by the controls for vector presence (3 consecutive negative samples).

Non-disposable materials, equipment and surfaces will be decontaminated by spraying with broad-spectrum disinfectants with proven activity against non-enveloped viruses. Solutions

such as Surfa'safe Spray (didecyldimethylammonium chloride) or Umonium (benzalkonium chloride, isopropilic alcohol and lauromyristic alcohol) may be used.

Any spillage produced will be collected following the procedure indicated in section V.D.2 and all surfaces will be decontaminated with the disinfectants indicated above.

The instructions and documentation for the destruction of the IMP and any waste generated will be followed and documented by a trained investigator at the study site.

The vector cannot be replicated, even in the presence of a helper virus, however, it can be present in the patient's excretions for a limited period of time after administration. For this reason, patients included in the study will be trained in the treatment of excretions that may contain the GMO, especially urine and feces. For this purpose, at least half a cup of sodium hypochloride (desinfectant bleach) will be added after each urine or faecal deposition by dropping it down the walls of the toilet before dumping it. Patients will wash their hands with soap after using the bathroom. This treatment will be continued until the vector's presence controls recommend so (3 negative consecutive samples). Similarly, male patients will refrain from sexual intercourse due to the potential presence of the GMO in the semen.

D. EMERGENCY RESPONSE PLANS

1. METHODS AND PROCEDURES FOR CONTROLLING THE GMOS IN CASE OF UNEXPECTED SPREAD

There are no specific procedures planned for controlling the GMO in the case of unexpected spread.

Wild type AAV is a non-pathogenic single-stranded DNA *Dependovirus*, requiring helper DNA virus for replication. AAV5-hFVIII-SQ is derived from wild type AAV, but encodes no replication genes in the expression cassette and is incapable of independently replicating its genome.

The potential for unexpected spread of AAV5-hFVIII-SQ in the environment is extremely low, due to:

- Attenuation of the GMO rendering it even less replication competent than the parental virus (AAV5), by deletion of the replication genes
- Intravenous administration to eligible patients by medical professionals in a medical facility.
- Limited host and tissue tropism (human/primate) of the parental virus (AAV5)
- Low and transient incidence of shedding of infective virus from treated individuals
- High levels of existing adaptive immunity in the human population

Any spread of AAV5-hFVIII-SQ to unintended human recipients is therefore highly unlikely, and would be isolated to single cases in discrete geographical locations. The risk of widespread infection is considered negligible.

In the theoretical event that wild type AAV, supplying the requisite replication gene products, were to co-infect a hepatocyte, along with a helper DNA virus such as adenovirus or herpes simplex virus and the AAV5-hFVIII-SQ vector (a triple co-infection), it is possible that vector replication could occur.

However, even if this rare event were to occur, the resulting virologic outcome would be synthesis of vector and wild type AAV, both intrinsically non-pathogenic viruses. It is therefore unlikely that such an event would present clinical symptoms and is therefore unlikely to become apparent.

The only foreseeable case of unexpected dissemination would be a spill during the preparation or administration of the product under study. This dissemination would always be contained within the room where the spill occurs. In this case, the instructions indicated in the following section will be followed.

2. METHODS FOR DECONTAMINATION OF THE AREAS AFFECTED, FOR EXAMPLE ERADICATION OF THE GMOS

The chance of dissemination of the vector is negligible outside of the contained pharmacy or the infusion room. Should the investigational product be spilled or otherwise dispersed during the preparation or administration the procedures in the Study Pharmacy Manual, distributed to the trial sites should be performed in accordance with standard practices for cleaning up biohazard waste spills, like those for treating potential blood borne pathogens.

For example as follows from the Pharmacy Manual:

- Notify others and isolate the area.
- If not already wearing, put on appropriate personal protective equipment: disposable aprons, gloves, particle protection facemask and safety glasses, face shield or goggles.
- Remove any broken glass or sharps with forceps or applicable tool and place into a sharps container.
- Decontaminate the area of the spill.
    - Place absorbent material over the spill.
    - Add disinfectant solution on the absorbing material and let it absorb the liquid.
    - Sweep up and place the absorbent material in infectious waste bag for disposal
    - Wash the area with broad-spectrum disinfectants with proven activity against non-enveloped viruses and dispose of all the used of all the used disposable materials as biological waste.

3. METHODS FOR DISPOSAL OR SANITATION OF PLANTS, ANIMALS, SOILS, ETC., THAT WERE EXPOSED DURING OR AFTER THE SPREAD

Decontamination of plants, (non-human) animals and soils will not be required.

4. METHODS FOR THE ISOLATION OF THE AREA AFFECTED BY THE SPREAD

There are no specific plans for isolation of an area should horizontal transfer occur between a patient receiving AAV5-hFVIII-SQ and an unintended human recipient.

5. PLANS FOR PROTECTING HUMAN HEALTH AND THE ENVIRONMENT IN CASE OF THE OCCURRENCE OF AN UNDESIRABLE EFFECT

In case of any incidents related to the GMO handling in Belgium, the information will be sent to the Belgian Biosafety Advisory Council within six month after the last visit of the last patient included into the study.

AAV5-hFVIII-SQ will be regulated under legislation in Belgium, requiring stringent pharmacovigilance overseen by the Federal Agency for Medicines and Health Products (FAMHP). Information will be collected regarding all individual adverse events and if they fulfil the criteria for a Serious Unexpected Suspected Adverse Reaction (SUSAR) as defined in the Clinical Trial Protocol. Development Safety Update Reports will be submitted to the FAMHP on an annual basis while the trial is active.

Information relating to trial-related monitoring activities is provided the protocol.

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