PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a) Member State of notification: Germany
(b) Notification number: B/DE/19/PEI3486
(c) Date of acknowledgement of notification: 09/07/2018
(d) Title of the project
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
(e) Proposed period of release
From November 2018 until April 2019

2. Notifier

Name of institution or company: BioMarin Pharmaceutical Inc.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)
Genus: *Dependovirus,*
Species: Adeno-associated virus/ serotype 5 (AAV 5)

(c) Genetic stability – according to Annex IIIa, II, A(10)

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. Genetic stability was demonstrated on three levels: stability of the vector genome sequence, stability indicated by functional protein production in vitro, and stability indicated by functional protein production in vivo.

DNA sequencing of AAV5-hFVIII-SQ demonstrated that vector genome integrity was maintained at the end of the manufacturing process. A cell based potency assay verified that BMN 270 makes functional human factor VIII in vitro, and mouse studies demonstrated that BMN 270 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. Homologous recombination may occur if a host organism is infected with wild type AAV plus a helper virus and BMN 270 Drug Product (DP), which would require a triple infection.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (X) No ()

If yes, insert the country code(s) ES

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (X) No ()

If yes:

- Member State of notification ES
- Notification number B/ES/18/11

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (X) No (.)

If yes:

- Member State of notification South Africa
- Notification number Not Applicable

7. Summary of the potential environmental impact of the release of the GMOs.

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 genetic modifications of AAV5-hFVIII-SQ do not affect its natural host and tissue tropism. No transfer of genetic material between the GMO and other organisms is predicted.
No specific studies have been conducted regarding transmission of AAV5-hFVIII-SQ between humans or animals.
Shedding will be monitored as part of the clinical trial.

The transfer of genetic material is 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 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).

There will be a single intravenous infusion of each study subject in the hospital setting of a dosing site: University Hospital Bonn, Institute for Experimental Haematology and Transfusion Medicine.

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 the S1 facility or in the infusion room of the phase I unit at the dosing site 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. There is a low probability that gene transfer could be made to other humans, however because the amount would be so small and the GMO is replication incompetent (even in the presence of helper virus) the result would be negligible.

Because 94% 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.

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

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	Dependovirus
(iii)	species	Adeno-associated virus
(iv)	subspecies	serotype 5 (AAV5)
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Adeno-associated virus

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes X

If yes, indicate the type of ecosystem in which it is found:

Atlantic	X
Mediteranean	X
Boreal	X
Alpine	X

	Continental	X
	Macaronesian	X
(ii)	No	(.)
(iii)	Not known	(.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	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, where it may remain inactive indefinitely, or be reactivated giving rise to secretion of virus.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques

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

1. Polymerase Chain Reaction (PCR).
2. Viral culture
3. Enzyme-Linked Immunosorbent Assay (ELISA) methods

(b) Identification techniques
See 5a

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes X No
If yes, specify
For Germany, the Central Committee on Biological Safety (ZKBS) has classified the AAV5-serotype as Risk Group 1. Genetic research with genetically modified organisms fulfilling the mentioned criteria is assigned to Containment Level 1.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No X Not known (.)

If yes:

(a) to which of the following organisms:

humans	(.)
animals	(.)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
AAV5 requires the co-infection of a helper virus so replication in an infected host can take from 24 to 48 hrs, but does not occur in the absence of an appropriate helper virus.

(b) Generation time in the ecosystem where the release will take place:
The AAV rep and cap gene coding sequences were removed from AAV5-hFVIII-SQ vector and cannot replicate even in the presence of a helper virus such as an adenovirus.

(c) Way of reproduction: N/A Sexual.. Asexual..

(d) Factors affecting reproduction:
The only way that an AAV5-hFVIII-SQ vector might be replicated would be in the presence of a helper virus, such as adenovirus, and a wild type AAV to provide the transacting *rep* and *cap* genes.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify AAV does not form survival structures, but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.	

(b) relevant factors affecting survivability:
Replication of wild-type AAV is dependent on co-infection of helper viruses such as adenovirus. AAV can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

10. (a) Ways of dissemination
AAV is thought to be spread in nature via inhalation of aerosolized droplets, mucous membrane contact or ingestion.

(b) Factors affecting dissemination
Environmental conditions which may affect survival of AAV5-hFVIII-SQ outside the host are temperature, pH and environmental humidity.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

No notification of genetic modifications of the recipient or parental organism has been notified by BioMarin Pharmaceutical Inc. in Germany

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	X
(ii)	deletion of genetic material	X
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
The outcome of the genetic modifications is to remove the rep and cap gene coding sequences, leading to the loss of replication ability, and the insertion of the human Factor VIII transgene expression cassette leading to the expression of functional hFVIII in the liver.

3. (a) Has a vector been used in the process of modification?
Yes X No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes X No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	X
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify …	

(b) Identity of the vector

The plasmid 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.

(c) Host range of the vector

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.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes X No (.)

antibiotic resistance (.)
other, specify Adenovirus sequences

Indication of which antibiotic resistance gene is inserted…

(e) Constituent fragments of the vector

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.

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …transduction

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert

The cassette sequence codes for a B domain-deleted human coagulation factor VIII, which is under the control of a liver-specific promoter, and is flanked by inverted terminal repeats.

(b) Source of each constituent part of the insert

The human FVIII is human in origin. The other sequences in the genome and promoter are synthetic, viral and mammalian in origin.

(c) Intended function of each constituent part of the insert in the GMO

The expression cassette is limited to the required elements designed to optimise expression of functional human coagulation Factor VIII under control of a liver-specific promoter.
The inverted terminal repeats are necessary for the packaging of the vector genome into the capsid and the formation of the episomal concatemers in the transduced cells.

(e) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: as episomal concatemers in the host cells

(f) Does the insert contain parts whose product or function are not known?

Yes (.) No X

If yes, specify …

D. Information on the organism(s) from which the insert is derived

The following information relates to the organism from which the inserted gene (hFVIII) is derived.

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals X
- insect (.)
- fish (.)
- other animal (.)

(specify phylum, class) …

other, specify …

2. Complete name

(i)	order and/or higher taxon (for animals)	…Primates
(ii)	family name for plants	…N/A
(iii)	genus	…Homo
(iv)	species	…*sapiens*
(v)	subspecies	…*sapiens*
(vi)	strain	…N/A
(vii)	cultivar/breeding line	…N/A
(viii)	pathovar	…N/A
(ix)	common name	…Human

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No X Not known (.)

If yes, specify the following:

(b) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.) No X Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No X

If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?

Yes X No (.) Not known (.)

At high multiplicity of infection, wild type AAV integrates into human chromosome 19 in ~60% of latently infected cell lines. However, it has been recently demonstrated that only approximately 1 out of 1000 infectious units can integrate (Tenenbaum *et al.*, 2003). Schnepp *et al.*, 2005 have provided evidence that following naturally acquired infection, wild type AAV DNA may persist mainly as circular double stranded episomes in human tissues.

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes (.) No X Not known (.)

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.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes X No (.) Unknown (.)

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.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes X No (.) Not known (.)

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. Therefore, though it has the capacity to infect cells, the lack of replicative capacity will severely restrict dissemination.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No X Not known (.)

Specify

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

2. Genetic stability of the genetically modified organism

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. Based on the fact that long term therapeutic activity of the investigational drug is not dependent on replication of the recombinant AAV, and the known genetic stability of the parent wild type AAV, the genetic traits of the organism are expected to be stable.
See also section A.3c

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No X Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

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

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

Polymerase chain reaction (PCR) based methods using vector genome specific primers can be used to detect GMO genetic elements.

(b) Techniques used to identify the GMO

Polymerase chain reaction based methods using vector genome specific primers can be used to detect GMO genetic elements.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

   The purpose of the release is 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.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

   Yes (X) No (.)

   If yes, specify:
   The replication incompetent GMO is administered intravenously and transient/low levels of vector DNA shedding is expected, however shed AAV-based vectors have been shown to be non-infectious.

3. Information concerning the release and the surrounding area

   (a) Geographical location (administrative region and where appropriate grid reference):

   The IMP dosing will take place at the central dosing facility

Site	**Investigator**
University Hospital Bonn, Institute for Experimental Haematology and Transfusion Medicine, Sigmund-Freud-Str. 25, 53127 Bonn	Prof. Dr. med. Johannes Oldenburg

The below sites will monitor (including handling of biosamples) the study patients after Day 1 of the BMN 270 administration at the central dosing facility:

Site	**Investigator**
Goethe-University Frankfurt, University Hospital, Medical Clinic II: Hematology and Hemostaseology, Theodor-Stern-Kai 7 60590 Frankfurt-am-Main	Prof. Dr. med. Wolfgang Miesbach
Vivantes Clinic in Friedrichshain, Center for Hemophilia and Hemostaseology, Landsberger Allee 49 10249 Berlin	PD. Dr. med. Robert Klamroth

   (b) Size of the site (m^2): … m^2
   (i) actual release site (m^2): Not applicable
   (ii) wider release site (m^2): Not applicable

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable considering that shed material, if any at all, is non-infectious.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
None.

4. Method and amount of release

(a) Quantities of GMOs to be released:

A maximum of 4 patients will be treated in Germany in Study 270-301, each receiving a single dose of BMN 270 at a dose of 6E13 vg/kg. Therefore, the maximum quantities of GMOs to be released is 1.92E16 vg, assuming an average subject weight of 80 kg.

(c) Duration of the operation:
The complete administration procedure including preparation of the infusion system is expected to take less than 8h.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Preparation of the investigational product will be done in a bio-safety laboratory classified for Risk Group 1. The administration of the investigational product will be made by authorized trained personnel at the study dosing site (University Hospital Bonn, Institute for Experimental Haematology and Transfusion Medicine) 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. Personnel handling the GMO will wear disposable apron, gloves, eye protection 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 AAV5/hFVIII.

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.

5. Short description of average environmental conditions (weather, temperature, etc.)

The clinical trial of AAV5-hFVIII-SQ will occur in Germany in treatment rooms with ambient indoor conditions.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

None.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	N/A
(iii)	genus	Homo
(iv)	species	*sapiens*
(v)	subspecies	*sapiens*
(vi)	strain	N/A
(vii)	cultivar/breeding line	N/A
(viii)	pathovar	N/A
(ix)	common name	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

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.
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.

3. Any other potentially significant interactions with other organisms in the environment

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 small and the GMO is replication incompetent (even in the presence of helper virus) the result would be negligible.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No X Not known (.)
Give details

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

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.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

Not applicable

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

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 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).

(b) from other organisms to the GMO:

As above.

(d) likely consequences of gene transfer:

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. The scenario described above 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).

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

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

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None known or predicted. AAV is not known to be involved in any biogeochemical 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.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Monitoring of the direct and indirect effects of BMN 270 in subjects will be achieved by the clinical assessments defined in the clinical trial protocol. 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 utilizing PCR.

2. Methods for monitoring ecosystem effects
No monitoring of the environment or unintended recipients is planned or considered necessary.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
PCR
However, it has been shown that the material from shedding is not infectious and thus transfer of donated genetic material from the patient to other organisms is not envisaged.

4. Size of the monitoring area (m^2)
Not applicable

5. Duration of the monitoring
Monitoring will occur throughout a subject's participation in the study, including a period of safety follow-up, as defined in the study protocol.

6. Frequency of the monitoring
Monitoring will be made according to the predefined schedule detailed in the study Protocol.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

All disposable materials (including but not limited to gloves, masks, syringes, needles, catheter and tubing) that come into contact with the investigational product will be disposed of as biohazardous materials according to individual institutional practices and policies. In general the materials will be disposed of in sharps containers or biohazard bags and decontaminated by incineration.

The unused investigational product and vials, stopper and crimp seal can be decontaminated with 3% Descogen solution and incinerated according to institutional practice. Following decontamination, materials will be disposed of as biohazardous waste. If excess investigational product is inactivated by chemical means it can be poured down a sink with running water or otherwise in compliance with local and institutional disposal and cleaning procedures.
Non-disposable materials, equipment and surfaces will be decontaminated with 3% Descogen.

2. Post-release treatment of the GMOs
Instructions for, and worksheets documenting the destruction of unused undiluted investigational product, along with associated generated waste will be followed and documented by the hospital staff in the dosing site. In general, treatment with 3% Descogen and incineration will be used for destruction of the GMO.

3. (a) Type and amount of waste generated

AAV5-hFVIII-SQ will be administered by a single intravenous infusion into eligible, consenting adult males with severe Haemophilia A.
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 potentially contaminated equipment to and from infusion room located in the phase 1 unit
• Used swabs and items used to clean injected area
• Personal Protective Equipment used during dose preparation and administration

3. (b) Treatment of waste

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.
Any unused or partially used BMN 270 should be retained at the dosing facility for investigational drug accountability and monitoring. BMN 270 may be destroyed at the dosing facility, according to the local operating procedures governing the destruction of genetically modified organisms. Any other disposable instruments or other materials used during the dose preparation procedure or biosamples collection will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) 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 negligible, 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 increased 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.

2. Methods for removal of the GMO(s) of the areas potentially affected

The chance of dissemination of the vector is negligible outside of the contained hospital pharmacy or lab. Should the investigational product be spilled or otherwise dispersed during the preparation or administration the procedures in the Study Pharmacy Manual, distributed to central dosing site should be performed in accordance with standard practices for cleaning up biohazard waste spills, like those for treating potential blood borne pathogens.
Accidental spills will be cleaned up according to standard local practice. For example as follows from the Pharmacy Manual:

- Notify others and isolate the area.
- If not already wearing, put on appropriate personal protective equipment: aprons, gloves, surgical or procedure mask and safety glasses, 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.
    - o Place absorbent material over the spill.
    - o Allow to stand for at least 5 minutes.
    - o Sweep up and place the absorbent material in infectious waste bag for disposal

- Wash the area with 3% Descogen and disposable materials

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   Decontamination of plants, (non-human) animals and soils will not be required.

4. Plans for protecting human health and the environment in the event of an undesirable effect

   AAV5-hFVIII-SQ will be regulated under medicines legislation in Germany, requiring stringent pharmacovigilance overseen by the Competent Authority (PEI). Information will be collected regarding all individual adverse events and submitted to the PEI 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 PEI on an annual basis while the trial is active.
   Information relating to trial-related monitoring activities is provided in the study protocol.