Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

# Advice of the Belgian Biosafety Advisory Council on the notification B/BE/19/BVW2 of the company BioMarin Pharmaceutical Inc. for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

24/05/2019
*Ref. SC/1510/BAC/2019_0491*

## Context

The notification B/BE/19/BVW2 has been submitted by BioMarin Pharmaceutical Inc. to the Belgian Competent Authority in January 2019 for a request of deliberate release in the environment of genetically modified organisms (GMO) other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a clinical trial involving two protocols, entitled "**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) and "**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). This multicentric clinical trial aims at investigating whether, in an expanded sample, the investigational medicinal product (IMP) can safely alter the clinical phenotype of hemophilia A patients with residual FVIII activity ≤ 1 IU/dL.

Hemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII) gene that codes for an essential cofactor in the coagulation pathway. This disorder can be either inherited or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, ultimately leading to a defective coagulation process. The clinical phenotype of HA patients generally correlates tightly with the level of residual FVIII expression. Treatment of severe HA presently consists of intravenous injection of plasma-derived or recombinant human FVIII protein (hFVIII) concentrates, both as prophylaxis 2-3 times per week, and at the time of a bleed, to prevent or control bleeding episodes, respectively. The short half-life for FVIII necessitates frequent infusions.
The experimental gene therapy approach planned with both studies aims at delivering the gene encoding hFVIII to the liver by single intravenous dose in order to achieve stable, potentially life-long expression of active hFVIII in the plasma, synthesized from vector-transduced liver tissue.

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The IMP BMN 270 (AAV5-hFVIII-SQ) is a disabled version of a non-pathogenic wild-type adeno-associated virus serotype 5 (AAV5), modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus. It contains an expression cassette with the human coagulation factor VIII (FVIII) gene under the control of a liver-specific promoter, leading to the expression of functional hFVIII in the liver.

A maximum of seven patients will be treated in Belgium in study 270-301, and up to three patients will be treated in study 270-302, each receiving a single dose of BMN 270 either at a dose of $6^{13}$ vg/kg (vector genome/kg) (study 270-301) or $4^{13}$ vg/kg (study 270-302). 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. Vector shedding will be monitored at several time points after administration utilizing PCR.

This study should be conducted in three clinical sites located in Brussels and the Flemish Region. The national territory is considered as the potential release area of BMN 270.

The dossier has been officially acknowledged by the Competent Authority on 22 February 2019 and forwarded to the Biosafety Advisory Council (BAC) for advice.
Within the framework of the evaluation procedure, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Two experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano answered positively to this request. The SBB also took part in the evaluation of the dossier.
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism (GMO) would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO) in the context of its intended use. See Annex I for an overview of all the comments from the experts.
The GMO laboratory of Sciensano evaluated the analytical procedure for the detection of BMN 270.

The scientific evaluation has been performed considering the following legislation:
- Annex II (principles for the risk assessment) and Annex III (information required in notifications) of the Royal Decree of 21 February 2005.
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.
The pure medical aspects concerning the efficacy of the IMP and its safety for the treated patient, as well as aspects related to social, economic or ethical considerations, are outside the scope of this evaluation.

On 5 April 2019, based on a list of questions prepared by the BAC, the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 3 May 2019 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator, and was considered satisfactory.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the above-mentioned Royal Decree. The Competent Authority did not receive reactions from the public.

## Summary of the scientific evaluation

### 1. The characteristics of the donor, the recipient or parental organism

The donor, recipient and parental organisms were found to be adequately described in the dossier.

### 2. Information related to the characteristics of the GMO and the medication

The molecular characteristics of BMN 270 including phenotypic and genetic stability of the transgene were found to be adequately described in the dossier.

### 3. The conditions of the release

BMN 270 will be delivered by single intravenous dose to adult subjects ≥ 18 years of age with hemophilia A and residual FVIII levels ≤ 1 IU/dL treated continuously with prophylactic exogenous FVIII for a minimum of one year prior to enrolment. An interim analysis is planned after approximately 26 weeks. The final analysis for the study will be performed after all subjects have been followed for 52 weeks post-BMN 270 infusion. After the final analysis, safety and efficacy will then continue to be assessed long-term in all subjects for a total of approximately five years.

The BAC takes notice that the current study will investigate vector shedding in blood, urine, semen, feces and saliva, at several time points after administration using polymerase chain reaction (PCR). Testing will be continued until at least three consecutive negative results are obtained. Testing of semen will continue through at least Week 12, even if three consecutive negative results have been obtained in this matrix prior to that time point. Subjects who have not had three consecutive negative semen samples by Week 52 will continue to have qPCR testing in semen every 4 weeks (during Year 2) and every 6 weeks (during Years 3-5) until three consecutive negative samples are documented.

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 the laboratory Good Microbiology Practices will be applied as a minimum for handling the GMO: the use of gloves, surgical masks 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.

### 4. The risks for the environment or human health

BMN 270 is a recombinant, replication deficient adeno-associated virus-based vector not harbouring any antibiotic or other resistance genes. Like its parental strain, it is not known to be pathogenic. The genetic modification introduced in this AAV5-derived vector does not confer the GMO with properties that could confer risks to the human population or the environment.

There is only a remote possibility of homologous recombination between the ITR-sequences of AAV5-hFVIII-SQ and wild-type AAV5 in case a triple infection by AAV5-hFVIII-SQ, wild type AAV (providing the rep and cap functions) and a helper virus occurs in exposed persons. Such recombination event would result in gain of functional genes of AAV5 required for replication and encapsidation but would in turn lead to the loss of the transgene. Moreover, the genetic material from *rep* and *cap* genes together with the transgene would be too large in size to be packed in AAV capsid, making it impossible to form a viral particle that would contain the transgene and the *rep* and *cap* genes necessary for multiplication. This scenario is expected to be a rare event, also because the vector target cells (liver) are not the natural target cells of helper viruses.

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' that may conceivably be transferred (from e.g. aerosol, splashing or fomites) will be orders of magnitude lower than that received by patients.

Since shedding of the IMP in the semen cannot be excluded, the notifier was asked to bring up arguments to determine the period in which use of a condom is absolutely necessary.
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 $6 \times 10^{12}$ vg/kg to $6 \times 10^{13}$ vg/kg administered to subjects at a central dosing facility. Data from this study show that, only low, steadily declining levels of BMN 270 are found in patient semen. Therefore, exposure to BMN 270 via semen is estimated to be very low, and lower than the sub-therapeutic exposure levels. In addition, given expression of FVIII-SQ is controlled by a liver-specific promoter, non-systemic exposure of low levels of BMN 270 are unlikely to produce protein.
The risk to the partner associated with exposure from semen of subjects after dosing of BMN 270 is therefore considered very low given: (a) non-replicating and non-pathogenic nature of the modified AAV5 vector, (b) low levels of BMN 270 found in patient semen, (c) the benign nature of the FVIII-SQ transgene and protein and (d) high level of post dose AAV5 antibodies in serum which are detectable at 2 weeks post-administration, and would be predicted to neutralize remaining capsids in the blood and seminal compartments.
As outlined in the inclusion criteria, all participants must agree to effective contraception use (which includes either double-barrier contraception [i.e., condom + diaphragm; or condom or diaphragm + spermicidal gel or foam] or their female partner either using hormonal contraceptives or having an intrauterine device) for at least 12 weeks post-infusion. After 12 weeks, subjects and/or their partners may stop effective contraception only if subjects have had three consecutive semen samples with no detectable viral vector DNA by week 12. Otherwise, effective contraception use remains obligatory until three consecutive negative semen results have been obtained.
The BAC concludes that, based on the above-mentioned arguments, the overall risk associated to exposure and transmission to other individuals can be considered negligible.

In the ongoing phase 1/2 clinical trial using BMN 270, long term (52 weeks) shedding was observed in blood, feces and semen samples. With respect to these observations, and following a request from the BAC, the notifier indicated that this shedding profile was not indicative of an insertion of the vector DNA in bone marrow (BM) stem cells. The concentrations of residual vector DNA in blood, stool and all other matrices evaluated continue to steadily decline over time. If integration into BM stem cells were to have occurred, a stable persistence or increase in vector DNA concentrations over time would be expected.

Persistence of low levels of residual transgene DNA in blood and feces may be associated with cells such as lymphocytes. T and B cells are long-lived lymphocytes that can harbor stable episomal DNA, which in turn could explain the persistence of low levels of transgene DNA in these tissues.
The BAC agrees with this analysis.

The applicant also indicated that integration site analysis using techniques such as LAM-PCR was not performed because the integration frequency of engineered AAV vectors is much lower than the spontaneous rate of mutation for human genomes so that the likelihood of insertional mutagenesis by AAV vectors is very low.

With regards to the 'Safety Data Sheet', the BAC notes that the description of the procedures for the management of accidental spills still leaves room for improvement and proposes to add the following procedure:
*In case of accidental spills, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 minutes. He/she should close the area and post a sign "DO NOT ENTER". After 30 minutes, he/she can enter the area wearing a lab coat and gloves, disposable overshoes, glasses and a FFP2 mask. He/she must cover the spill with towels and other absorbent material starting from the edge toward the center. He/she must carefully pour the appropriate disinfectant over the absorbent material starting from the edge to the center. It must allow a sufficient contact time for the disinfectant to inactivate the GMO. After that, he/she must remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag. The PPEs should be discarded in the biohazard bag. The lab coat should be decontaminated before disposal. The medical staff should report the incident to the responsible of the site.*

**5. The monitoring, control, waste treatment and emergency plans proposed by the applicant**

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.
Given that the likelihood of further propagation of BMN 270 can be considered highly unlikely, the BAC supports the view that, in terms of risk for the environment or human health, the proposed measures are proportionate and adequate in the context of the intended trial.

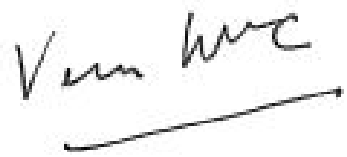
## Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that BMN 270 developed to treat hemophilia A patients with residual FVIII activity, will have any adverse effects on human health or on the environment in the context of the intended clinical trial and provided that all the foreseen safety measures are followed.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the clinical trial protocol and the safety instructions as described in the dossier.
- The document 'Safety data Sheet' should be updated as mentioned under section 4 above.

- Any protocol amendment has to be previously approved by the Competent Authority.
- The notifier is responsible to verify that each study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorisations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on the contained use of genetically modified micro-organisms.
- The BAC should be informed within two weeks when the first patient starts the treatment and the last patient receives the last treatment.
- At the latest six months after the last visit of the last patient included in the trial, the notifier must send the competent authority for the attention of the BAC a report with details concerning the biosafety aspects of the project. This report shall contain at least:
  - The total number of patients included in the trial and the number of patients included in Belgium;
  - A summary of all adverse events marked by the investigators as probably or definitely related to the study medication;
  - A report on the accidental releases, if any, of BMN 270.



Dr. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

***Annex I:*** *Compilation of comments of experts in charge of evaluating the dossier B/BE/19/BVW2 (ref. SC/1510/BAC/2019_0269)*

# Compilation of comments of experts in charge of evaluating the dossier B/BE/19/BVW2

4 April 2019
*Ref. SC/1510/BAC/2019_0269*

**Mandate for the Group of Experts:** Mandate of the Biosafety Advisory Council (BAC) of 19 November 2018.
**Coordinator:** Anton Roebroek (KUL)
**Experts:** Aline Baldo and Amaya Leunda (Sciensano, SBB), Viggo Van Tendeloo (UZA), Willy Zorzi (ULiège)
**SBB:** Didier Breyer

## INTRODUCTION

Dossier **B/BE/19/BVW2** concerns a notification from BioMarin Pharmaceutical Inc. for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 22 February 2019.
It concerns a clinical trial involving two protocols, entitled "*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) and *"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), respectively.

## ♦ INSTRUCTIONS FOR EVALUATION

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

## Comments/questions received from the experts

### 1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

*Comment 1*

Has not evaluated this item.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

### 2. INFORMATION RELATED TO THE VECTOR

(e.g. description, sequence, mobilisation ...)

*Comment 1*

Has not evaluated this item.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

### 3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

#### 3.1. Information related to the genetic modification

(e.g. methods used for the modification, description of the insert/vector construction ...)

*Comment 1*

Has not evaluated this item.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

### 3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

*Comment 1*

Has not evaluated this item.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Given the high dose rAAV administered especially in the 207-302 study ($4.10^{13}$ vg/kg) and knowing that the average integration frequencies of rAAV in human livers are 1 in 1000, more precisely approx. $1.17 \times 10^{-3}$ integrations per cell occurring via non homologous recombination (Gil-Farina et al, Mol Ther. 2016; 24(6): 1100–1105), it is therefore incorrect to state that the frequency of integration will be several orders of magnitude below the spontaneous rate of mutation in human cells. The notifier should rephrase this in their SNIF and technical dossier NCI (Annex IIIa) p. 21.

***Comment coordinator:***

*An average integration frequency of rAAV in human livers is 1 in 1000, more precisely approx. 1.17 × 10−3 integrations per cell occurring via non homologous recombination (52 weeks after vector infusion, 5 x $10^{11}$ and 6 x $10^{12}$ vg/kg) (Gil-Farina et al, Mol Ther. 2016; 24(6): 1100–1105) is not contradicting the statement. Recently the mutation rate per base pair per mitosis for a human somatic cell was estimated to be 2.66 x $10^{-9}$.(Milholland et al. Nat Commun. 2017; 8:15183. doi: 10.1038/ncomms15183). Given a size of 3.2 Gbp or 3.2 x $10^{9}$ bp per haploid nuclear genome this implies that two livers cells differ at least in one of more basepair positions depending on the number mitoses that they have underwent since their descent of a common ancestor cell.*

### 3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

1) The BMN 270 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. The potential for unexpected spread of AAV5-hFVIIISQBMN 270 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

2) 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).

3) 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.

Expert comment: On basis of these 3 points, we consider the risk for the human and animal health and the environment as being negligible

*Comment 3*

Has evaluated this item and has no questions/comments.

## 4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment ...)

*Comment 1*

The applicant describes in the dossier the personal protective equipments (PPE) used for the infusion of the IMP to the patients. The medical staff wears a surgical mask. This type of mask is not considered as a PPE. The medical staff should wear a FFP2 mask for the infusion of the IMP to the patients.

In case of accidental spills, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 min. He/she should close the area and post “DO NOT ENTER”. After 30 min, he/she must wear a clean lab coat and wear gloves, glasses and a FFP2 mask. He/she must cover the spill with towels and other absorbent material starting from the edge toward the center. He/she must carefully pour the appropriate disinfectant over the absorbent material starting from the edge to the center. It must allow a sufficient contact time for the disinfectant to inactivate the GMO.

After that, he/she must remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag. The PPE should be discarded in the biohazard bag. The lab coat should be decontaminated before disposal. The medical staff should report the incident to the responsible of the site.

***Comment SBB and coordinator:***

*The same comment was formulated in the final advice of the Council for dossiers B/BE/18/BVW4 and B/BE/18/BVW9. Agreement to include this comment in the final advice of the Council as a recommendation to update the "Safety Data Sheet".*

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

## 5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

### 5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

*Comment 1*

Shedding of the IMP in the semen cannot be excluded. Consequently, the male patients will refrain from sexual intercourse. The applicant could also propose the use of a condom as an alternative.
The patients who receive the IMP cannot give their blood until the clearance of the GMO and cannot give organs.

***Comment coordinator:***

*The requirement (in 03a_ICF_Main_Dutch, page 3/4): "Er worden onder meer spermastalen genomen om zeker te weten dat het studiegeneesmiddel niet in uw sperma aanwezig is (om mogelijke problemen voor uw partner en voor een kind dat u en uw partner verwekken te voorkomen). U moet dan ook een effectieve anticonceptiemethode gebruiken gedurende ten minste 12 weken na toediening van het studiegeneesmiddel en tot 3 opeenvolgende spermastalen negatief zijn (wat tot een jaar of langer na de infusie kan duren)." is indeed inadequate with respect to protection of the partner. In fact the same accounts for the differently worded requirement in the Clinical Protocol (page 10): "Sexually active participants must agree to use an acceptable method of effective contraception, either double barrier contraception (ie, condom + diaphragm; or condom or diaphragm + spermicidal gel or foam) or their female partner either using hormonal contraceptives or having an intrauterine device. Participants must agree to contraception use for at least 12 weeks post-infusion; after 12 weeks, subjects may stop contraception use only if they have had 3 consecutive semen samples with no detectable viral vector DNA."*

*The applicant should require to use anyhow always additionally a condom during an initial period after infusion until it can be expected, that no remaining viral particles will be shed in the semen, but only cell-associated episomal vector DNA present in purified sperm cells and non-sperm cells in the semen (see also comment by coordinator on comment immediately below). Since a PCR analysis cannot distinguish DNA from a remaining viral particle and from an episomal vector DNA in the semen the applicant is asked to bring up arguments to determine the period in which use of a condom is absolutely necessary. Subjects who received the IMP should be advised to abstain livelong from any blood, sperm or organ donation.*

*Comment 2*

Given the nature of the product administration (intravenous) and the transient/low levels of shedding expected, the risk of unintended exposure to BMN 270 for 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.
None of the vector genomes are detected in sperm so there is no likelihood of vertical transmission.

Expert comment: concerning this point, we consider the risk for the human health as being negligible.

*Comment 3*

Given the intravenous administration and the lack of specific liver-targeting moieties on the vector capsid proteins (use of a human liver promoter only ensures transgene expression in the liver but is not per se associated with liver-specific targeting properties and hepatocyte-specific transduction), vector shedding of rAAV in body fluids as well as potential vertical transmission of the vector through transduction of gametes should carefully be checked, especially since the phase I study revealed persistent presence of rAAV in the sperm of one of the study participants (Annex IIIa p.23).

***Comment coordinator:***

*With respect to the previous clinical trials using AAV5-Factor VIII (BMN 270) it is hypothesized in the supplementary discussion on shedding, that white blood cells in semen (typically present at ~ 1 Mio/mL) carry the vector DNA and are responsible for the signal obtained in seminal fluid after week 26 (Rangarajan et al. N Engl J Med. 2017;377(26):2519-2530). These white blood cells may have been transduced during vector infusion, due to the high vector dose used, either through active uptake of the vector via endocytosis or weak tropism of AAV5 vectors for mononuclear cells. The clearance kinetics of cell-associated episomal vector DNA, in semen, blood, or any other compartment, would thus follow the turn-over rate of the affected cells, rather than the expected rapid clearance dynamics of vector capsids from plasma.*
*Thus most likely, the long term (52 weeks) shedding observed in blood, feces and semen samples (Rangarajan et al., N Engl J Med. 2017;377(26):2519-2530) reflects cell-associated episomal vector DNA. According to figure 4 (Rangarajan et al., N Engl J Med. 2017;377(26):2519-2530) the median levels of vector DNA in the blood and feces in the high-dose cohort drop initially, but tend not to reach a negative value. With respect to these observations, the applicant is asked to comment on the possible explanation that insertion of the vector DNA in BM stem cells is the source of a potentially persisting presence of the vector DNA in blood and feces? An average integration frequency of rAAV in human livers was determined to be 1 in 1000, more precisely approx. 1.17 × 10−3 integrations per cell occurring via non homologous recombination (52 weeks after vector infusion, 5 x $10^{11}$ and 6 x $10^{12}$ vg/kg) (Gil-*

*Farina et al, Mol Ther. 2016; 24(6): 1100–1105). Integration could also happen in BM stem cells. If follow-up analysis would further point to persisting presence vector DNA in blood and feces as consequence of vector integration does the applicant foresee integration site analysis using an technique like LAM-PCR to detect integrated viral DNA sequences?*

### 5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

*Comment 1*

The applicant does not consider the risk for immunocompromised people.

***Comment coordinator:***

*To my knowledge, AAV is not known to be pathogenic in humans, including immunocompromised or immunosuppressed people (see also comment for item 5.5).*

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

### 5.3. Information on possible effects on animal health or on the environment.

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

For this point, please consider the evaluation of the point 3.3. of this document

*Comment 3*

Has evaluated this item and has no questions/comments.

### 5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

### 5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

There is a small but realistic chance that either the rAAV vector (vector mobilization) or reconversion to the wild type AAV (virus rescue) might occur when the vector transduced cell (hepatocyte or other cells) also contains wild type AAV sequences (trans acting *rep* and *cap* genes) from previous infections (note: AAV infections are quite abundant though not pathogenic in the general population) and when sur-infection with a helper virus such as adenovirus of the same cell takes place. However, in numerous previous trials with rAAV, there has been no proof of such (recombination) events nor adverse effects from liver-targeting rAAV gene transfer (except transiently elevated liver enzymes due to cellular immune responses towards the vector).

**Comment coordinator:**

This a highly relevant question/remark. Indeed according to Hüser at al. (Virology 91 (4): e02137-16, 2017), the high prevalence of infectious AAV in human peripheral blood mononuclear cells is indicative of T lymphocytes as sites of AAV persistence, which is even increased in immunosuppressed people. From these sites of AAV persistence reactivation and rescue by helper viruses can occur. Only in case the rAAV (BMN 270) would transduce such a cell, the REP and CAP functions could be provided in order to mobilize the vector. For mobilization, however, additional helper virus functions will be needed for transcription of the *rep* and *cap* genes from the wildtype AAV. So an additional infection by a helper virus will be needed. Recombination between rAAV and the wildtype AAV would most likely result in two replication-defective viral genomes or alternatively in another wildtype-like AAV and another replication-

deficient virus as the capacity to encode genes in a replication-competent virus will be completely occupied by the *rep* and *cap* genes. Also in these cases a helper virus will be needed for mobilization of the recombinants. In the numerous previous trials with rAAV there has been no proof of such adverse effects occurring, probably due to the fact, that the likelihood of combination of the three requirements happening is too low. Increasing vector doses make coinfection of cells with pre-existing wild-type AAV, however, more likely, which may result in unwanted mobilisation of the rAAV. Therefore, careful surveillance is needed to monitor the shedding of rAAV during long follow-up studies as is the case for the current protocol.

### 5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

### 5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

## 6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

### 6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Given the intravenous administration and the lack of specific liver-targeting moieties on the vector capsid proteins (use of a human liver promoter only ensures transgene expression in the liver but is not per se associated with liver-specific targeting properties and hepatocyte-specific transduction), vector shedding of rAAV in body fluids/secretions as well as potential vertical transmission of the vector (risk to offspring) through transduction of gametes should carefully be checked, especially since the phase I study revealed persistent presence of rAAV in the sperm of one of the study participants (Annex IIIa p.23).

***Comment coordinator:***

*Same comment was addressed at item 5.1*

### 6.2. Surveillance and control of the release

(adequation between the procedures to avoid and/or minimise the spread of the GMO and risks identified during the risk assessment...)

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

**6.3. Information on the waste generated by the activity and its treatment.**
(e.g. type of waste, amount ...)

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

**6.4. If applicable, information on the emergency plan(s) proposed by the notifier.**

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

**6.5 Information related to the identification of the GMO and the detection techniques**
(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

*Comment 1*

Has not evaluated this item.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Will the notifier also use LAM-PCR to detect (integrated) proviral sequences ?

***Comment coordinator:***

*See item 5.1*

## 7. OTHER INFORMATION

### 7.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

*Comment 1*

None

*Comment 2*

None

*Comment 3*

There is a small semantic error in the “ICF Pregnancy Dutch” document. First sentence in ‘Inleiding’ : ‘gevraag’ should be ‘gevraagd’

**References**

Gil-Farina *et al.* (2016). Recombinant AAV Integration Is Not Associated With Hepatic Genotoxicity in Nonhuman Primates and Patients. Mol Ther., 24(6): 1100–1105

Hüser et al. (2017) High prevalence of infectious adenoassociated virus (AAV) in human peripheral blood mononuclear cells indicative of T lymphocytes as sites of AAV persistence. Virology 91 (4): e02137-16

Milholland *et a*l. (2017) Differences between germline and somatic mutations rates in humans and mice. Nat Commun. 8:15183. doi: 10.1038/ncomms15183

Rangarajan *et al.* (2017). AAV5-factor VIII gene transfer in severe hemophilia A. N Engl J Med. 377(26):2519-2530