Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

# Advice of the Belgian Biosafety Advisory Council on the notification B/BE/18/BVW6 of the company AveXis, Inc. for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

07/01/2019
*Ref. SC/1510/BAC/2019_0004*

## Context

The notification B/BE/18/BVW6 has been submitted by AveXis, Inc. to the Belgian Competent Authority in October 2018 for a request of deliberate release in the environment of genetically modified organisms (GMOs) other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned project concerns two clinicals trials with the following titles *" AVXS-101-Cl-302: Phase 3, Open-Label, Single-Arm, Single-Dose Gene Replacement Therapy Clinical Trial for Patients With Spinal Muscular Atrophy Type 1 With One or Two SMN2 Copies Delivering AVXS-101 by Intravenous Infusion'* and '*AVXS-101-CL-304: A Global Study of a Single, One-Time Dose of AVXS-101 Delivered to Infants with Genetically Diagnosed and Pre-symptomatic Spinal Muscular Atrophy with Multiple Copies of SMN2".*

Spinal muscular athropy (SMA) is an autosomal recessive neuromuscular disorder which occurs in one out of 8000-10000 children and is caused by a loss or mutation in the survival motor neuron 1 gene (*SMN1*), leading to reduced SMN protein levels and selective dysfunction of motor neurons. Men carry two *SMN* genes (*SMN1* and *SMN2*) and disease severity and clinical prognosis, ranging from the most severe SMA type I to milder SMA type IV, depends on the number of copies of *SMN2*. *SMN2* generally produces between 10 to 20% of the fully functioning SMN protein compared to the primary *SMN1* gene, due to a splicing defect in *SMN2* that largely excludes exon 7. The gene replacement therapy approach planned with both studies aims at treating pre-symptomatic patients with the delivery of the *SMN1* gene by means of intravenous administration of the investigational medical product (IMP), AVXS-101. The primary objectives of the Phase III AVXS-101-Cl-302 study is to investigate the efficacy of AVXS-101 in patients with SMA type I and at less than 6 months of age at the time of AVXS-101 infusion. The primary objective of the second global study *AVXS-101-CL-304* is to demonstrate the safety and efficacy of AVXS-101 in patients of less or equal than 6 weeks of age at time of dose.

AVXS-101 is a recombinant, non-replicating and non-integrating self-complementary adeno-associated virus serotype 9 (AAV9) capsid shell containing the cDNA of the *SMN* gene as well as two inverted repeats from the AAV serotype 2 (AAV2) DNA.
Patients will receive a one-time dose of AVXS-101 at 1.1 X $10^{14}$ vector genome/kg and may be discharged 24 hours after the infusion based on Investigator judment. Following dosing, follow-up visits

will be conducted every week for the first four weeks and at Month 2 and Month 3 followed by every 3 months. No viral shedding analysis is foreseen.

It is planned to conduct the trial in clinical sites located in the Flemish and the Walloon Region. Up to 10 patients are anticipated to be enrolled in the proposed clinical studies .

The dossier has been officially acknowledged by the Competent Authority on 16 October 2018 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 Biosafety and biotechnology Service (SBB) of Sciensano answered positively to this request and two members of the BAC took part in the evaluation of the dossier.
The experts 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 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 scientific evaluation has been performed considering 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 medicinal product 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 03 December 2018, 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 13 December 2018 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts.
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 abovementioned 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

Upon request of additional information the notifier clarified that according to their expression analysis the *SMN* cDNA clone encodes a cDNA that contains the "C -T" base transition that is normally associated with a disrupted splicing of exon 7. But the cDNA used to construct the vector correctly includes exon 7, therefore leading to the production of a fully functional SMN protein. The Biosafety Advisory Council is of the opinion that the characteristics of the *SMN* cDNA clone does not affect the evaluation of AVXS-101 with respect to possible risk for human health or environment. Information related to the molecular characteristics of AVXS-101 including phenotypic and genetic stability of the transgenes were further adequately described in the dossier.

## 3. The conditions of the release

AVXS-101 will be administered intravenously to infants with genetically diagnosed SMA at multiple sites globally. After administration of $1.1 \times 10^{14}$ vector genome/kg, shedding of vector DNA in urine, saliva or faeces is expected to last for a few weeks after administration. Detection of vector genomic material was perfomerd during a Phase I study with 5 patients showing levels of 10.0 – 100.0% of the dosing concentration up to 14 days post-dose in stool with concentrations declining approximately 4 logs over 30 days post dose. Levels in stool below the limit of quantitation were observed by 60 days post dose. It should be noted that the technique used to detect vector genomic material (ddPCR) does not allow to distinguish between vector genomic material or intact virus, hence the observed shedding data may not correctly reflect the shedding of intact infectious viral particles. The amounts of shed intact infectious viral particles is likely to be much lower.

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

AVXS-101 is a recombinant, replication deficient adeno-associated virus-based vector not harbouring any antibiotic or other resistence genes. Like its parental strain it is not known to be pathogenic. It also does not elicit a strong immune response. The genetic modification introduced in this AAV9/AAV2-based vector does not confer the GMO with properties that could confer risks to the human population or the environment.

While shedding of vector DNA in urine, saliva or faeces is expected for a few weeks after administration, there are no data available on the amount of shed infectious particles. A study in a nonhuman primate by Favre *et al.*[1] showed that rAAV vector genome was found in various biological fluids for up to 6 days and infectious particles exclusively in the serum during the first 48–72 hours. In its response the notifier also referred to a review (Schenk-Braat, 2007)[2] reporting that 'in non of seven studies of AAV-based therapies intact viral shedding was detected in human bodily fluids'. It can be reasonably assumed that the amount of shed infectious particles will be extremely low and only a minute fraction of the applicable dose. Given the horizontal transfer, the shedded levels can reasonably be considered as non-infectious. Taken together with the fact that material shed will be replication-deficient, the likelihood of further propagation of the GMO should be considered highly unlikely. The BAC concludes that the overall risk associated to exposure and transmission to other individuals can be considered as negligible.

There is only a remote possibility of homologous recombination between the ITR-sequences of AVXS-

[1] Favre *et al*. Immediate and Long-Term Safety of Recombinant Adeno-associated Virus Injection into the Nonhuman Primate Muscle. Molecular Therapy 4, 559-566, 2001

[2] Schenk-Braat EA *et al*., An inventory of shedding data from clinical gene therapy trials. J Gene Med. 2007 Oct ç(10): 910-21. Review.

101 and wild-type AAV2 in case a co-infection with AAV2 occurs in exposed persons. Such recombination event would result in gain of functional genes of AAV2 required for replication and encapsidation but would in turn lead to the loss of the transgene. It is also remarked that 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.

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

The Biosafety Advisory Council raised several concerns with respect to the Handling Instructions' for the patient's family (document 2017- 004087-35 - Handling Instructions_V1.0_05Jun2018) and asked the notifier i) to prepare a more detailed and fit-for-purpose instruction sheet identifying the type of fluids that are potentially contaminated and how to properly dispose them ii) to address the potential risks for other children living in the home, in particular when they are young enough to not have completely matured immune systems yet (under 6 years old), and iii) to follow these special handling instructions until stool tests are negative for vector DNA and they are given the all clear from the study center. In its response the notifier focused primarily on the characteristics of AVXS-101 (which is incapable of generating a productive infection even in the presence of environmentally occurring helper viruses) and the observed 4 logs reduction at 30 days of shed vector genomic material. On this basis the notifier considers its proposed decontamination procedures sufficient and additional testing of stool unnecessary.
Given that the likelihood of further propagation of AVXS-101 can be considered highly unlikely, the Biosafety Advisory Council 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 expert, the Biosafety Advisory Council concludes that it is unlikely that AVXS-101 developed to treat paedriatic infants with SMA will have any adverse effects on human health or on the environment in the context of the intended clinical trial 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 all the safety instructions as described in the dossier.

- 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 authorizations 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 Contained use of genetically modified micro-organisms.

- The Biosafety Advisory Council should be informed within two weeks when the first patient starts the treatment and the last patient receives the last treatment.

- The notifier has to deliver to Sciensano - Transversal activities in applied genomics a control sample the latest 15 days after the approval and the start of the trial.

- At the latest six months after the last visit of the last patient included in the trial, the notifier must send to the competent authority at the attention of the Biosafety Advisory Council a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - 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 AVXS-101.

Dr. Corinne Van der Wauven
President of the Belgian Biosafety Advisory Council

**Annex I:** *Compilation of comments of experts in charge of evaluating the dossier B/BE/18/BVW6 (ref. SC/1510/BAC/2018_1033)*

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

# Compilation of comments of experts in charge of evaluating the dossier B/BE/18/BVW6 and comments submitted to the notifier

03 December 2018
*Ref. SC/1510/BAC/2018_1033*

**Mandate for the Group of Experts:** Mandate of the Biosafety Advisory Council (BAC) of 26 September 2018.
**Coordinator:** Karen Willard-Gallo (ULB)
**Experts:** Anton Roebroek (KUL), Liliane Tenenbaum (CHUV), Willy Zorzi (Ulg), Aline Baldo (Sciensano, SBB)
**SBB:** Katia Pauwels.

## INTRODUCTION

Dossier **B/BE/18/BVW6** concerns a notification of the company AveXis, Inc. for 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 12 October 2018 and concerns a global study of a recombinant adeno-associated virus designed to address the monogenic root cause of Spinal Muscular Atrophy. The biosafety dossier includes two clinical studies (two protocols).

## ♦ 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.

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## List of comments received from the experts

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 03-12-2018 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

**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 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.

*Comment 4*

Has evaluated this item and has no questions/comments.

**2. INFORMATION RELATED TO THE VECTOR**

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

*Comment 1*

Has evaluated this item and has no questions/comments.

*Comment 2*

Has not evaluated this item.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

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 evaluated this item and has no questions/comments.

*Comment 2*

Has not evaluated this item.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

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*

Table in SNIF (page 12 of 23) and Table 1 in Annex IIIA (page 11 of 30) mention with respect to the human SMN cDNA 'Genbank Accession #NM_017411 (one nucleotide difference in relevant region)'. What is the nature of the one nucleotide difference? A silent variation with respect to the open reading frame ?

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

Has not evaluated this item.

### 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*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

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*

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.

*Comment 4*

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*

The SNIF (page 18 of 23) states '*In the Phase 1 study all five patients analyzed were dosed with 2E14 vg/kg. Concentrations of vector shed in saliva and urine are quite low and are below the limits of quantitation by ddPCR in the matrices within days post dose. While initially concentrated in stool, the amount of vector shed declines logarithmically. Levels of 10.0 – 100.0% of the dosing concentration are detectable up to 14 days post-dose in stool. These concentrations decline approximately 4 logs over 30 days post dose, and all patients had levels of AVXS 101 in stool below the limit of quantitation by 60 days post dose. Levels representing 0.1−0.01% of the initial dose into the patient are found in urine and saliva at 1 day post dosing, after which levels of AVXS-101 shed into these matrices are below the limit of quantitation of the assay. Together these data demonstrate rapid decline of shed vector quantities well below dosing concentrations in patients treated with AVXS 101*.'.
What is exactly the significance of '10.0 – 100.0% of the dosing concentration' in relation to an injected dose of 2E14 vg/kg? Apparently, 100.0% should not be interpreted as shedding of all vector, which has been injected.
Furthermore, as shedding quantification is based upon the shedding of viral vector DNA, the question remains whether infectious viral particles are shed. According to a study in a nonhuman primate by Favre *et al*., 2001 rAAV vector genome was found in various biological fluids for up to 6 days and infectious particles exclusively in the serum during the first 48–72 hours. If extrapolated to humans this could indicate that shedding of infectious viral particles via e.g. the stool is not occurring at all, except maybe for the first days. With respect to this, are data available about shedding of infectious AVXS-101 viral particles by patients of the Phase 1 study confirming this?

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

Has evaluated this item and has no questions/comments.

***Comment Coordinator :***

The applicant states that there are 3 potential mechanisms of spreading the GMO into the environment: 1) needle stick accident, 2) blood (needle stick injury) or 3) shedding from the patient.
Then they dismiss it as negligible. I think they should be required to address this issue in detail. This goes with what Willy says below.

Furthermore, it is totally unnecessary to double bag the vial and the syringe used to administer the GMO AND RETURN IT TO THE COMPANY – It should be correctly disposed of in the biohazard waste at the hospital administering the GMO. Keeping it around for later return to the company just creates an increased risk of inadvertent release and serves no purpose other than their silly need to control that it was actually injected.

### 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*
Has evaluated this item and has no questions/comments.

*Comment 2*

Has evaluated this item and has no questions/comments.

*Comment 3*

In this dossier, AVXS-10 will be administered intravenously to infants with genetically diagnosed Spinal Muscular Atrophy at multiple sites globally.

In the ERA dossier, it is written that '*AVXS-101 GMO is non-pathogenic and the human SMN protein is not known to have toxic effects. No side-effects have been reported for the environment or human health after the release of similar GMOs (adeno-associated virus from serotypes 2 and 9). AAV2 and AAV9 are non-pathogenic, -toxigenic, -virulent, -allergenic nor a carrier (vector) of a pathogen. Vector shedding can be found in the urine, saliva, and stool for up to a few weeks (four) following injection. The risks associated with the shed vector are not known at this time; however, they are unlikely to be a problem as the vector is non-infectious and cannot replicate.*

*Regardless, instructions should be provided to patient families and care givers regarding use of protective gloves if/when coming into direct contact with patient bodily fluids and/or waste as well as good hand-hygiene for four weeks after the injection. Additionally, patients are prohibited from donating blood for two years following the vector injection'*.

The patients, after their treatment will go back home. GMO vector shedding can be found in blood, urine, saliva and stool for up to four weeks following injection (after gene replacement therapy). At home, they will use their own toilet, bathroom, etc., in contact with other members of their family (children, pets), close people, people coming to their house… In the SNIF dossier and in the ERA dossier, the only instructions for the families and care givers are to use protective gloves if/when come into contact with patient bodily fluids and/or waste, as well as to have a good hand-hygiene. Insufficient information is provided concerning the decontamination of the patient room (SOP? Provisioning of a disinfectant kit? …). There is also the question of the use of public toilets (open or restricted?) by the treated patients, eventually the indirect exposure of people (healthy or potentially immuno-suppressed?) using these facilities thereafter, etc. Considering that the treated patients have to return home during periods without clinical support and that the patients are having, at this time, direct or indirect contact with the population, there is a problem in the risk assessment.

The risks associated with the shed AVXS-101 vector are not known at this time; however, they are unlikely as this GMO vector is non-infectious and cannot replicate. When the patients are back home, no detailed treatment is considered to treat shed viruses in faeces discharged into the sewage system. Could it be sufficient that the subsequent dilution by flushing toilet, and waste water treatment will substantially reduce virus concentrations down to an insignificant level? Is this dilution procedure sufficient to accept such a GMO release in the environment ?

***SBB comment :***

The applicant states that '*The risks associated with the shed vector are not known at this time; however, they are unlikely to be a problem as the vector is non-infectious and cannot replicate',* however the applicant fails to further substantiate why it can be concluded that the shed vector is non-infectious. See comment 1 under section 5.1.

*Comment 4*

Has evaluated this item and has no questions/comments.

***Comment Coordinator:***

I have looked thorough all the documents to find information or instructions for the parents or home care providers. There is very little. In the informed consent form, there is a small paragraph embedded in a huge amount of information on the risks to the child and what is expected from them during the trial. I believe that most parents are quite desperate to find something that will help their child with SMA and so will not pay careful attention to all these "risk" details and in the process will overlook the little available information on how they need to handle the potentially contaminated fluids, particularly in the first days and weeks after injection.

Babies drool saliva, spit up milk and other food, tear frequently when they cry, and of course have dirty diapers. The person(s) handling the baby needs to know the risks and how to properly clean up these fluids and dispose of the waste.

A detailed instruction sheet identifying the type of fluids that are potentially contaminated and how to properly dispose of them (adding a little "eau de javel" to a dirty diaper or wipe before double bagging it would seem to be simple and easy way to reduce the risk of spread into the environment since this waste will go untreated into a landfill somewhere. The applicant could even provide plastic squeeze bottles labelled with the appropriate dilution).

Handling Instructions' for patient's family (document 2017-004087-35 - Handling Instructions_V1.0_05Jun2018) is woefully inadequate! A detailed instruction sheet for the family that the applicant needs to prepare must be provided to every parent and medical personal should go over it with them carefully before the patient goes home so they clearly understand the risks to them and others living in the home or who will come in close contact with the patient. Also, There is no mention of other children living in the home and the potential risks to them, particularly if they are young enough to have incompletely mature immune systems (around 6 years old).

### 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*

Has evaluated this item and has no questions/comments.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

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.

*Comment 4*

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*

Has evaluated this item and has no questions/comments.

*Comment 4*

Has evaluated this item and has no questions/comments

**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.

*Comment 4*

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.

*Comment 4*

Has not evaluated this item.

## 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*

Has evaluated this item and has no questions/comments.

*Comment 4*

Has evaluated this item and has no questions/comments

### 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.

*Comment 4*

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*

As it is not clear, for how long infectious virus particles are shed, a positive analysis for shedding of viral DNA should be interpreted as potential shedding of infectious viral particles. Therefore, it might not be sufficient to restrict the 'AVXS-101 - Handling Instructions' for patient's family (document 2017-004087-35 - Handling Instructions_V1.0_05Jun2018) to only the first four weeks after AVXS-101 treatment, because shedding of viral DNA can apparently continue up to 60 days post dose, especially in stool. Shedding of viral DNA, including in stool, will be monitored for 30 days (in AVXS-101-CL-302). As long as monitoring of shed viral DNA remains positive, monitoring should be continued after 30 days and patient's family should continue to apply the special handling instructions.
With respect to trial AVXS-101-CL-304 it is not clear, why viral DNA shedding is monitored no longer at all?
'AVXS-101 - Handling Instructions' (document 2017-004087-35 - Handling Instructions_V1.0_05Jun2018) should be made available in Dutch and French.

***Comment Coordinator:***

Standards to protect the family and the environment must be maintained until the stool test is negative.

*Comment 2*

Have evaluated this item and has no questions/comments.

*Comment 3*

The expert refer to comment 3 under 5.2.

*Comment 4*

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 not evaluated this item.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

Has not evaluated this item.

**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 evaluated this item and has no questions/comments.

*Comment 2*

Has not evaluated this item.

*Comment 3*

Has evaluated this item and has no questions/comments.

*Comment 4*

Has evaluated this item and has no questions/comments.

## 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*
None

*Comment 4*

The very high dose of vector (10e14 vg/kg) needed to efficiently transduce neurons when using intravenous injections is a concern. This protocol could result in a very strong immune response directed against AAV9 capsid proteins (Gray SJ ***et al***., Gene Therapy (2013) 20, 450–459).
However, this is more a concern for the patient than for the environment. Furthermore a Phase I trial has established the safety of this protocol.

## References

Favre *et al*. Immediate and Long-Term Safety of Recombinant Adeno-associated Virus Injection into the Nonhuman Primate Muscle. Molecular Therapy 4, 559-566, 2001