

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/18/PEI3354*  
(c) Date of acknowledgement of notification *02.03.2018*  
(d) Title of the project:  
*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.*  
(e) Proposed period of release: *From 1H 2018 until 2 H 2021*

2. Notifier

Name of institution or company: *AveXis, Inc.*  
*2275 Half Day Road, Suite 200*  
*Bannockburn, IL 60015, United States*

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

other, specify (kingdom, phylum and class)

*Recombinant adeno-associated vector serotype 9 (AAV9) containing human Survival Motor Neuron (SMN) gene (AVXS-101) for the treatment of Spinal Muscular Atrophy (SMA).*

(b) Identity of the GMO (genus and species)

*Family: Parvoviridae*

Genus: *Dependovirus*

Species: *Adeno-associated Virus (AAV9-derived replication-deficient viral vector containing human SMN cDNA)*

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

*The genetic stability in regard to the genetic traits is expected to be equivalent to the wild type AAV. DNA of wild type AAV and of AAV-based vectors persists in transduced cells as circular (extrachromosomal) episomal concatemers in human tissues (Chen et al. 2005, Penaud-Budloo et al. 2008, Schnepf et al. 2005, Schnepf et al. 2009). However, due to the lack of viral Rep and Cap genes, AVXS-101 is expected to remain in the cells as episomes and will not replicate and produce viral particles. The expression cassette will be transcribed and translated by host cell enzymes leading to expression of SMN.*

*In general, DNA viruses have greater genetic stability than RNA viruses. This may be attributed to factors such as (a) DNA being more thermodynamically stable than RNA, (b) replication of DNA being much less error-prone process than the replication of RNA and (c) more mechanisms exist in the host cell for repairing errors in DNA than in RNA. Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus.*

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

Yes  No

If yes, insert the country code(s)

BE

DE

ES

FR

GB

IT

NL

SE

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

Yes  No

If yes:

- Member State of notification GB

- Notification number CESP ID: 647702

MHRA Ref: 46842/0001/001-0001

**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  No

If yes:



(specify phylum, class)

other, specify

2. Name

- |       |   |                               |
|-------|---|-------------------------------|
| (i)   | order and/or higher taxon (for animals) | <i>Family of Parvoviridae</i> |
| (ii)  | genus                                   | <i>Dependovirus</i>           |
| (iii) | species                                 | <i>Adeno-associated virus</i> |
| (iv)  | subspecies                              | <i>N/A</i>                    |
| (v)   | strain                                  | <i>N/A</i>                    |
| (vi)  | pathovar (biotype, ecotype, race, etc.) | <i>Serotype 2 and 9</i>       |
| (vii) | common name                             | <i>AAV2/9</i>                 |

3. Geographical distribution of the organism

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

- (b) Indigenous to, or otherwise established in, other EC countries:

- (i) Yes

*Approximately 50 to 80% of the European human population is seropositive to at least one AAV serotype.*

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

- |               |                                     |
|---------------|-------------------------------------|
| Atlantic      | <input checked="" type="checkbox"/> |
| Mediterranean | <input checked="" type="checkbox"/> |
| Boreal        | <input checked="" type="checkbox"/> |
| Alpine        | <input checked="" type="checkbox"/> |
| Continental   | <input checked="" type="checkbox"/> |
| Macaronesian  | <input checked="" type="checkbox"/> |

- (ii) No

- (iii) Not known

- (c) Is it frequently used in the country where the notification is made?

- Yes  No

- (d) Is it frequently kept in the country where the notification is made?

- Yes  No

4. Natural habitat of the organism

- (a) If the organism is a microorganism

- |   |                          |
|---|--------------------------|
| water                                       | <input type="checkbox"/> |
| soil, free-living                           | <input type="checkbox"/> |
| soil in association with plant-root systems | <input type="checkbox"/> |
| in association with plant leaf/stem systems | <input type="checkbox"/> |

other, specify

*Human*

*AAV has been isolated from human and non-human primates, although other animals can be hosts. Specifically, AAV9 has been isolated from human tissues.*

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

5. (a) Detection techniques  
*Droplet digital PCR or ddPCR with primers and probe specific to SMN gene in AVXS-101*
- (b) Identification techniques  
*Droplet digital PCR (ddPCR) with primers and probe specific to SMN gene in AVXS-101, Western blot techniques*

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?  
Yes (.) No (X)

If yes, specify

*Wild type AAV is not classified in Risk Groups 2,3, or 4 in the European Union (EU) according to directive 2000/54/EC on protection of workers from risks related to exposure to biological agents at work (Appendix III). It is most appropriately designated a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'. The only viral sequences included in the vector construct for AVXS-101 are the ITRs of AAV2, which are required for both viral DNA replication and the packaging of the rAAV vector genome. This makes AVXS-101 unlikely to cause disease in humans.*

*Similar classifications of hazard have been assigned to wild type AAV according to the definitions of the World Health Organisation (WHO) WHO Laboratory Biosafety Manual 2004, and in the US NIH Recombinant DNA guidelines 2016.*

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  
*Although human infections are common, AAV is not known to be a pathogenic virus in humans and has never been implicated as an etiological agent for any disease (Tenenbaum et al., 2003). Almost no human innate immune response is seen in AAV*

*infection (Zaiss et al., 2002) and at the adaptive level it is primarily made up of a humoral response. Pre-existing antibodies in patients, because of prior infection, account for the humoral response seen toward AAV. Cell-mediated responses to AAV vectors have been documented, but this response may be dependent on the route of administration. Despite the lack of evidence for pathogenicity, correlations have been made between: (i) Presence of AAV viral DNA sequences in testicular tissue and abnormal semen samples (Rohde et al., 1999), (ii) the occurrence infectious AAV in embryonic material as well as in the cervical epithelium (Burguete et al., 1999). A clear association is hard to establish from these studies, given that co-incident evidence of human papillomavirus infection is present in most subjects, and that AAV DNA can be detected in cervical samples in the majority of women (Burguete et al., 1999) but is very dependent on differences in sample collection between studies (Erles et al., 2001). An additional, theoretical, risk of AAV infection is the risk of insertional mutagenesis caused by non-site specific integration of the AAV genome into the host-cell genome of infected cells. Preclinical data indicate that in most cases, DNA delivered by recombinant AAV vectors predominantly persists as extrachromosomal elements (episomes) rather than integrating into host cell genomes (McCarty, et al., 2004). Although AVXS-101 is also not anticipated to integrate into the host cell genome as described above, the long-term consequences of administering AAV viral vectors to humans are not yet fully understood. This is in contrast to wild-type AAV, also non-pathogenic, which has the ability to stably integrate into the host cell genome at a specific site (designated AAVS1) in the human chromosome 19 (Kotin, et al., 1990; Surosky, et al., 1997).*

*Since the AVXS-101 product uses AAV9 with all of the wild-type DNA removed from the capsids, except for the Inverted Terminal Repeats, the potential risk of incorporation of AVXS-101 into the patient chromosomal DNA is thought to be significantly reduced.*

*There are conflicting reports that integration of the wild-type AAV2 genome is associated with induction of hepatocellular carcinoma in a small subset of patients.; however there are several studies with evidence to contradict these claims including; a) AAV2 has infected approximately 90% of the human population, b) AAV2 has been shown to possess anticancer activity, c) epidemiological evidence suggests that AAV2 infection plays a protective role against cervical carcinoma, and d) AAV serotypes including recombinant AAV2 and AAV9 have been or are currently used in 162 clinical trials to date in which no cancer of any type has been observed or reported (Srivastava and Carter, 2017).*

*Further support for the extremely low potential incorporation into host chromosomal DNA comes from pre-clinical studies, which to date have not shown the development of cancer in treated animals including mice and non-human primates exposed to AVXS-101.*

*It is possible the AAV9 vector containing the SMN gene could interact with other viruses with which the patients come in contact, such as rhinoviruses, adenovirus, or herpes. If this happens, the AAV9 vector could form a virus that causes infection if the patient and cells for rescue, replication, and packaging are also exposed to wild-type AAV2. The rescue, replication and packaging would stop; however, as the helper viruses, such as rhinoviruses, adenovirus, or herpes were cleared by the patient's immune system. This unlikely scenario has been studied. In cell culture, the rAAV genome can be rescued and replicated by superinfection with wtAAV and a helper virus. However, in vivo rescue experiments have failed to show rescue and replication (Favre et al., 2001), except in one case in which very large doses of*

*wtAAV and adenovirus were administered in a particular setting (Afione et al., 1996). Therefore, AAV9 interaction with other viruses to cause infection appears to be a minimal risk for AVXS-101.*

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

*The replication of AAV2 and AAV9 in an infected host is dependent on co-infection with a helper virus such as adenovirus and the generation time of wild-type AAV in a natural ecosystem will vary from weeks to years, depending on the timing of the co-infection. Generation time for AVXS-101 is not relevant since it lacks all viral replicative genes.*

(b) Generation time in the ecosystem where the release will take place:

See (a)

(c) Way of reproduction:                      Sexual                      ..                      Asexual                      X

(c) Factors affecting reproduction:

*Permissive host cell, reproduction of wild-type AAV is dependent on co-infection with helper virus (such as adenovirus or herpesvirus)*

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

*Upon infection of cells by AAVs, their genomes form concatemers that remain episomal for extended periods of time.*

(b) relevant factors affecting survivability:

*AAV is a non-enveloped virus that is relatively stable in the environment and stable to desiccation. AAV is sensitive to appropriate viricidal disinfectants, such as 1000 PPM chlorine solution. Replication of wild-type AAV is dependent on co-infection of helper viruses such as adenovirus or herpes-simplex virus. In presence of helper virus, AAV undergoes productive infection characterized by genome replication, viral gene expression and virion production. In absence of a herpes virus co-infection, the virus DNA will persist within infected cells in episomal form or may integrate into the host cell genome. In both cases the virus remains latent.*

10. (a) Ways of dissemination

*Dispersal (dissemination) of AAV is not documented definitively, but is likely*

*through inhalation (aerosolized droplets), contact with mucous membranes (eyes, nose and mouth) faecal-oral transmission.*

- (b) Factors affecting dissemination  
*Co-infection with a helper virus.*

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)  
*None*

**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

*AVXS-101 is a recombinant biological product that is comprised of a non-replicating, non-integrating recombinant self-complementary adeno-associated virus serotype 9 (AAV9) capsid shell containing the cDNA of the human SMN gene under the control of the cytomegalovirus (CMV) enhancer/chicken- $\beta$ -actin-hybrid promoter (CB) as well as two AAV inverted terminal repeats (ITR) from the AAV serotype 2 (AAV2) DNA. The left AAV ITR has been modified to promote intramolecular annealing of the transgene, thus forming a double-stranded transgene ready for transcription. This modified ITR, termed a “self-complementary” (sc) ITR, has been shown to significantly increase the speed at which the transgene is transcribed and the resulting human SMN protein is produced. Recombinant scAAV can be employed for AVXS-101 because of the small size of the SMN gene, which enables efficient packaging and allows for efficient gene transfer with lower viral titers, compared with prototypical single-stranded AAV vectors. All of the DNA from the wild-type AAV9 has been removed and replaced with the genes described above (the two ITRs are from AAV2). These modifications render AVXS-101 incapable of replicating itself which may be considered a potential safety benefit, when compared to integrating vectors with the ability to replicate, in that the total dose of virus administered to a patient can be carefully controlled and there is minimal risk of unintended transmission.*

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 vector construct contains the SMN complementary deoxyribonucleic acid (cDNA) expression cassette flanked by AAV2 inverted terminal repeat (ITR) sequences. The pSMN plasmid containing the vector construct was constructed by inserting the human SMN mRNA sequence into plasmid pAAV-MCS, which contains the CMV enhancer / CB promoter and uses the Simian virus 40 (SV40) intron for high-level expression and the bovine growth hormone (BGH) polyadenylation (poly A) termination signal. The only viral sequences included in this vector construct are the ITRs of AAV2, which are required for both viral DNA replication and the packaging of the rAAV vector genome. A modification to the “left” ITR allows for the production of self-complementary genomes (McCarty 2008). This sequence was encapsidated into AAV9 virions.*

(c) Host range of the vector

*Bacterial cells*

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

Yes	(X)	No	(.)
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antibiotic resistance (X)

other, specify

Indication of which antibiotic resistance gene is inserted

*There are no antibiotic resistance genes in AVXS-101. However, kanamycin resistant genes are present in the plasmids used in the manufacturing process of AVXS-101.*

(d) Constituent fragments of the vector

*AVXS-101 drug substance is produced by 3 plasmid DNA transfections.*

***pSMN Plasmid***

*The AAV vector genome derived from pSMN is a self-complementary DNA genome, with AAV2 derived inverted terminal repeats (ITR) flanking the expression cassette. Deletion of the Rep binding site within 5' ITR allows for the production of self-complementary genomes (McCarty, et al., 2008). The ITRs are the only viral sequences included in this vector, which are required for viral DNA replication and packaging of the rAAV vector genome. The gene cassette is under the control of the CMV enhancer/chicken-β-actin-hybrid promoter derived from plasmid pAAV-MCS. The 16S late intron from SV40 is included for efficient gene expression, as is the bovine growth hormone polyadenylation termination signal. These 2 elements were PCR amplified from plasmid pscAAV.CMV.GFP-. The human survival motor neuron*

*cDNA corresponding to the mature mRNA, was cloned into the self-complementary AAV vector plasmid derived from pAAV-MCS. Full sequencing of the encapsidated AAV vector DNA was completed.*

***pAAVPlasmid***

*The pAAV plasmid is an AAV helper plasmid that encodes the 4 wild-type AAV rep proteins from serotype 2 and the 3 wild-type AAV VP capsid proteins from serotype 9. The identity of the AAV9 capsid (Cap9) gene was confirmed by DNA plasmid sequencing and restriction digest map for each MCB and WCB.*

***pHELP Adenovirus Helper Plasmid***

*Plasmid pHELP is 11,635 base pairs (bp) in size. The plasmid contains the regions of adenovirus genome that are important for AAV replication, namely E2A, E4, and VA RNA (the adenovirus E1 functions are provided by the HEK293 cells), but does not contain other adenovirus replication or structural genes. The adenovirus sequences present in this plasmid represent approximately 28% (9,280 / 35,938) of the adenovirus genome, and do not contain the cis elements critical for replication such as the ITRs. Therefore, no infectious adenovirus is expected to be generated from such a production system. The identity of these 3 adenovirus genes were confirmed by DNA sequencing on the plasmid source stock. DNA Analysis revealed 100% homology with the 3 Adenovirus type 5 gene regions.*

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

- (i) transformation (.)
- (ii) electroporation (.)
- (iii) macroinjection (.)
- (iv) microinjection (.)
- (v) infection (.)
- (vi) other, specify *transfection*

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 vector construct contains the SMN complementary deoxyribonucleic acid (cDNA) expression cassette flanked by AAV2 inverted terminal repeat (ITR) sequences. The pSMN plasmid containing the vector construct was constructed by inserting the human SMN mRNA sequence into plasmid pAAV-MCS, which contains the CMV enhancer / CB promoter and uses the Simian virus 40 (SV40) intron for high-level expression and the bovine growth hormone (BGH) polyadenylation (poly A) termination signal. The only viral sequences*

included in this vector construct are the ITRs of AAV2, which are required for both viral DNA replication and the packaging of the rAAV vector genome. A modification to the “left” ITR allows for the production of self-complementary genomes (McCarty 2008). Through the AVXS-101 manufacturing process, this vector construct sequence is encapsidated into AAV9 virions.

**Figure 1: Vector Construct**



(b) Source of each constituent part of the insert

**Table 1 Position of features of the DNA sequence for plasmid AVXS-101**

Component	Description	Purpose
"Left" Mutated AAV2 Inverted Terminal Repeat (ITR)	McCarty modification to the "left" ITR by deleting the terminal resolution site to allow hairpin formation of genome	Produce second-generation self-complementary (sc) vector to maximize vector potency, allowing lower systemic doses
Cytomegalovirus (CMV) Enhancer / Chicken- $\beta$ -Actin Hybrid (CB) Promoter	Portion of the CMV immediate/early enhancer Chicken- $\beta$ - actin core promoter	Constitutive high-level SMN expression
SV40 Intron	Intron from the simian virus 40 (shown to enhance accumulation of steady level of mRNA for translation)	Common feature in gene vector for increased gene expression
Human SMN cDNA	Genbank Accession #NM_017411 (one nucleotide difference in relevant region)	Express full-length SMN protein
Bovine Growth Hormone (BGH) Polyadenylation (Poly A) Termination Signal	BGH poly A signal	Efficient polyadenylation of the SMN mRNA (transcription termination signal) for high-level, efficient gene expression
"Right" AAV2 ITR	Unmodified AAV2 ITR	Required in <i>cis</i> for both viral DNA replication and packaging of the rAAV vector genome

(c) Intended function of each constituent part of the insert in the GMO  
See table above in (b)

(e) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify

*The insert described is the entire AVXS-101 vector genome and encodes the human SMN protein. The location of the insert in the host organism will be mainly extrachromosomal by formation of episomal concatemers.*

(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

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) *Primate*
- (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 (.) No (.) Not known (.)

*Not applicable as the transfer is a human protein (SMN) to humans*

#### **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 (.)

Specify:

*AVXS-101 virus particles have similar ex vivo survival characteristics in the open environment as wild type AAV9, as the AVXS-101 capsid particle is similar to that of AAV9.*

(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 (.)

Specify:

*Due to the removal of the rep and cap genes AVXS-101 is unable to reproduce/replicate even in the presence of wild-type AAV helper virus (Adenovirus)*

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

Yes (.) No (X) Not known (.)

Specify:

*The viral capsid proteins have the same dissemination/tropism as the parent AAV9 virus. However, since AVXS-101 cannot replicate, the dissemination is limited to the administration of the AVSX-101 to the patient.*

(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 AVXS-101 are pathogenic to humans or the environment. Moreover, since AVXS-101 cannot replicate, it cannot enter an infectious cycle even in the presence of helper function.*

2. Genetic stability of the genetically modified organism

*All tests for identity, purity, and quality have confirmed the stability of AVXS-101. Upon administration to human subjects, AVS-101 infects target cells, but no new virus particles are being formed. In the cell, multiple AVXS-101 genomes assemble to form larger double stranded DNA concatemers. These concatemers persist in the cell as stable episomal structures and are transcriptionally active. In absence of an intrinsic mechanism for the genetic variation or instability and based on the known genetic stability of the wild type AAV, the genetic traits of AVXS-101 are expected to be stable.*

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)

*Annex III A, point II(A)(11)(d). Preclinical data indicate that in most cases, DNA delivered by recombinant AAV vectors predominantly persists as extrachromosomal elements (episomes) rather than integrating into host cell genomes (McCarty, et al., 2004). Although AVXS-101 is also not anticipated to integrate into the host cell genome as described above, the long-term consequences of administering AAV viral vectors to humans are not yet fully understood. Since the AVXS-101 product uses AAV9 with all of the wild-type DNA removed from the capsids, except for the Inverted Terminal Repeats, the potential risk of incorporation of AVXS-101 into the patient chromosomal DNA is thought to be significantly reduced.*

*It is possible the AAV9 vector containing the SMN gene could interact with other viruses with which the patients come in contact, such as rhinoviruses, adenovirus, or herpes. If this happens, the AAV9 vector could form a virus that causes infection if the patient and cells for rescue, replication, and packaging are also exposed to wild-type AAV2. The rescue, replication and packaging would stop; however, as the helper viruses, such as rhinoviruses, adenovirus, or herpes were cleared by the patient's immune system. This unlikely scenario has been studied. In cell culture, the rAAV genome can be rescued and replicated by superinfection with wtAAV and a helper virus. However, in vivo rescue experiments have failed to show rescue and replication (Favre et al., 2001), except in one case in which very large doses of wtAAV and adenovirus were administered in a particular setting (Afione et al., 1996).*

*Therefore, AAV9 interaction with other viruses to cause infection appears to be a minimal risk for AVXS-101.*

*AVXS-101 has been studied in one clinical study (AVXS-101-CL-101) including 15 patients; a full understanding of all risks is not known at this time.*

*Patients could experience an allergic response to AVXS-101. Patients are likely to develop an immune response to the AAV9 viral vector, which could interfere with or prevent future use of gene transfers using AAV9 as a viral vector.*

*Some mice affected with a form of SMA Type 1 that were treated with the study vector developed localized vascular necrosis around the ear called necrotic pinna. This is believed to be unrelated to the vector, and likely related to an underlying defect that has been observed to occur in several SMA mouse models (Narver et al. 2008). The relevance to humans with spinal muscular atrophy is unknown.*

*Some mice affected with SMA Type 1 that were treated with AVXS-101 experienced changes in liver function enzymes and also tiny deterioration and repair of tissues in the heart and liver; the heart and liver changes were visible only by a microscope.*

*AVXS-101 will likely express SMN protein in many different cell types in addition to motor neurons. While expression of SMN protein in many different cell types is not currently associated with any negative impact, all consequences are not known at this time.*

*Elevated liver function tests have been observed in AVXS-101-CL-101 trial participants, which is believed to reflect a T-cell immune response to the AAV9 vector. In some cases, the elevated liver function tests may become serious adverse events. In general, prolonged liver enzyme elevation can be an indication of clinically significant liver damage. However, none of the liver enzyme abnormalities observed in the AVXS-101-CL-101 study were accompanied by clinical symptoms, and all cases to date have resolved with generally short-term prednisolone treatment.*

*II(C)(2)(i) AVXS-101 is a non-replicating vector and the administration of AVXS-101 to patients is associated with limited exposure of the environment to AVXS-10. Thus, exposure of plants or animals is not expected. AVXS-101 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). Vector shedding can be found in the blood, urine, saliva, and stool for up to a few weeks following injection. The risks associated with the shed vector are not known at this time; however, it is unlikely 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 a few weeks after the injection. Additionally, patients are prohibited from donating blood for two years following the vector injection.*

#### 4. Description of identification and detection methods

- (a) Techniques used to detect the GMO in the environment  
*Droplet digital PCR or ddPCR with primers and probe specific to SMN gene in AVXS-101. This technique however is only applicable where sufficient DNA can be recovered for analysis.*
- (b) Techniques used to identify the GMO

*Droplet digital PCR or ddPCR with primers and probe specific to SMN gene in AVXS-101, Enzyme-Linked ImmunoSpot (Elispot) – A T-cell response to AAV will be detected via blood. Enzyme-Linked Immunosorbent Assay (ELISA)- AAV antibodies will be detected via ELISA from serum*

**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 clinical study designed as an open label, single arm, single-dose gene replacement therapy for patients with SMN Type I with one or two SMN2 copies. No environmental benefit is expected.*

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

*AVXS-101 will be administered intravenously to SMN Type I patients at multiple sites. Shedding of vector DNA in urine, saliva or faeces can be expected afterwards for a few weeks, 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):

*Universitätsklinikum Freiburg  
Zentrum für Kinder- und Jugendmedizin  
Klinik für Neuropädiatrie und Muskelerkrankungen  
Mathildenstr. 1  
79106 Freiburg*

*Dr. von Haunersches Kinderspital ISPZ  
Kinderklinik und Kinderpoliklinik der Ludwig Maximilians Universität München  
Lindwurmstr. 4  
80337 München*

*Universitätsklinikum Essen  
Klinik für Kinderheilkunde I  
Hufelandstrasse 55  
45147 Essen*

- (b) Size of the site (m<sup>2</sup>): N/A

- (i) actual release site (m<sup>2</sup>):  
(ii) wider release site (m<sup>2</sup>):

- (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:

*Up to 30 patients are anticipated to be enrolled in the proposed Phase 3 open-label, single arm, multi-center clinical study. At a given site, it can be expected up to 5 patients will receive a proposed dose. Patients will receive a one-time dose of AVXS-101 at  $1.1 \times 10^{14}$  vg/kg, a dose determined to be equivalent to the dose received by the Cohort 2 patients in the Phase 1 study (AVXS-101-CL-101) by direct testing using improved analytical methods. The appropriate number and size of vials will be determined for each patient based on body weight as well as product titer for the specific AVXS-101 product lot.*

*The quantities that will be released into the environment by shedding will be a very small proportion of the total number of viral genomes injected, of which the majority, if not all, is not infectious. AVXS-101 is detectable in the shed samples from day 1 post injection. 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.*

(b) Duration of the operation:

*The complete administration procedure including preparation of the infusion system is expected to take less than 2 hours.*

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release:

*All involved personnel on the site 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 involves, among others, wearing adapted protective clothing, gloves and goggles, the constant presence of a spill kit and the decontamination of waste prior to disposal.*

*Personal Protective Equipment (PPE) should include:*

- *Gloves (consider double-gloving)*
- *safety goggles*
- *lab coat*
- *Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.*

- *Personnel should not work with AAV, if skin is cut or scratched.*

5. Short description of average environmental conditions (weather, temperature, etc.)  
*The clinical trial will be performed at up to 16 investigational sites in Europe. The preparation and injection of AVXS-101 will take place in environmentally controlled hospital rooms (ambient indoor conditions for administration). AVXS-101 will be shipped frozen and stored at 2–8°C prior to administration.*

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.  
*A Phase I clinical trial for AVXS-101 (AVXS-101-CL-101) is currently ongoing in the United States. The goal of AVXS-101 therapy is to increase the expression level of the SMN protein in motor neurons prior to the development of irreversible injury and motor neuron loss, thereby modifying the patient's SMA phenotype to a milder course with improved quality of life and prolonged survival. A clinical study (AVXS-101-CL-101) in Type 1 patients dosed at <9 months of age has demonstrated prolonged survival with 100% of patients surviving beyond 13.6 months of age, the timepoint at which only 25% of patients survived in the recent natural history data (Finkel et al. 2014). As of 20 Jan 2017, all patients have experienced improvement in motor function relative to their baseline assessment, as measured by The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders, or CHOP INTEND, a test developed to measure motor skills of patients with SMA Type 1. Additionally, patients in the proposed therapeutic dose cohort consistently achieved and maintained key developmental motor milestones that are essentially never observed in the natural history data such as head support, rolling, sitting with and without support, standing, and walking independently (De Sanctis et al. 2016). AVXS-101 appears to have a favorable safety profile and to be generally well tolerated. In the most recent cut-off data (7 August 2017), AVXS-101 continues to show a favorable safety and tolerability profile and further improvement in efficacy. As of 7 August 2017, patients treated with AVXS-101 continue to demonstrate improved nutritional status including lower instances of feeding support (e.g., G-tube, NJ tube), improvement in motor function and achievement of developmental milestones such as sitting unassisted. Key efficacy findings related to motor function (when all treated patients reached 20 months of age or older post gene therapy) and safety data description can be found in the article by Mendell et al., 2017.*

*It is important to highlight that AVXS-101 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). Vector shedding can be found in the urine, saliva, and stool for up to a few weeks following injection. The risks associated with the shed vector are not known at this time; however, it is unlikely 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 a minimum of two weeks after the injection.*

*Additionally, patients are prohibited from donating blood for two years following the vector injection.*

**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)	<i>Primate</i>
(ii) family name for plants	<i>N/A</i>
(iii) genus	<i>Homo</i>
(iv) species	<i>sapiens</i>
(v) subspecies	<i>sapiens</i>
(vi) strain	<i>N/A</i>
(vii) cultivar/breeding line	<i>N/A</i>
(viii) pathovar	<i>N/A</i>
(ix) common name	<i>Human</i>
  
2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
 

*In treated subjects, AVXS-101 can cross the blood brain barrier allowing an IV infusion dosing route and effective targeting of both central and systemic features. AVXS-101 specifically targets motor neurons providing a rapid onset of effect. The vector DNA is expected to persist in transduced cell by the formation of episomal concatemers.*
  
3. Any other potentially significant interactions with other organisms in the environment.
 

*None expected for this product.*
  
4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
 

Yes	(.)	No	(X)	Not known	(.)
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Give details  
*AVXS-101 cannot replicate and, therefore, its dissemination or selection is not expected.*
  
5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
 

*Although AVXS-101 could theoretically infect the cells of other mammals, it cannot initiate a productive replication cycle because it is replication incompetent and therefore does not establish in other ecosystems.*
  
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:

*The likelihood of genetic exchange to other organisms in the release ecosystem is negligible. AVXS-101 is a non-replicating vector and the administration of AVXS-101 to patients is associated with limited exposure of the environment to AVXS-101. Even if AVXS-101 is released into the environment through shedding, due to the low numbers of vector DNA copies, horizontal gene transfer is highly unlikely. Even if horizontal gene transfer occurred, the sequences would not confer a selective advantage to other organisms such as bacteria since AVXS-101 does not contain any prokaryotic promoters, any antibiotic- or other types of resistance genes or any genes, which would enhance or constrain their growth. Therefore, it is unlikely that AVXS-101 would have an effect on the natural dynamics of microbial populations or the biogeochemical cycles at any given site in the environment.*

(b) from other organisms to the GMO:

*Negligible. Since AVXS-101 contains the ITR-sequences of AAV2, there is a (remote) possibility of homologous recombination of the vector with wild type AAV2 in case of a co-infection in exposed persons. The result of such a recombination would be that AVXS-101 would gain functional genes of the AAV2 required for replication and encapsidation, but, in turn, would lose the transgene. Hence, recombination would lead to the formation of viruses that are identical to the starting material and replication incompetent.*

(c) likely consequences of gene transfer:

*Expression of hSMN protein.*

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 references available.*

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

*None known or predictable since wild type AAV is not known to be involved in any biogeochemical process.*

## **H. Information relating to monitoring**

1. Methods for monitoring the GMOs

*AVXS-101 can be monitored by ddPCR assay using primers and probe specific to the SMN gene.*

2. Methods for monitoring ecosystem effects

*No monitoring is considered necessary.*

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

*The likelihood of transfer of AVXS-101 to other organisms is negligible and no monitoring is planned. The existing ddPCR assay used in the non-clinical and clinical studies could be used.*

4. Size of the monitoring area (m<sup>2</sup>)  
*Not applicable. Only subject's body fluids will be monitored after administration.*
5. Duration of the monitoring  
*The biological (saliva, urine, and stool) samples will be collected for viral shedding studies and monitored for 30 days.*
6. Frequency of the monitoring  
*The biological samples will be collected for viral shedding studies at 24 hours post-dose, 48 hours post-dose, Day 7, Day 14, Day 21, and Day 30.*

**I. Information on post-release and waste treatment**

1. Post-release treatment of the site  
*After administration of AVXS-101 to the subject, the procedure room will be cleaned according to standard institutional procedures.*
2. Post-release treatment of the GMOs  
*Since AVXS-101 will be supplied by the manufacturer to the hospital pharmacy in a subject-by-subject manner, no unused product should remain at the hospital center after administration of the patients. Any open vials or unused material must be sealed in leak-proof containers and returned to AveXis/designated depot.*
3. (a) Type and amount of waste generated  
*Empty vials and used vials and the used delivery system components (guide tube, cannula, injection needles and syringes), gauzes, personal protective equipment (e.g. gloves etc) and components used for collecting body fluids samples after administration.*
3. (b) Treatment of waste  
*After administration of AVXS-101 to the subject, all materials used for injection, including sterile drapes, needles, and syringes in contact with the vector must be sealed in leak-proof containers. All waste must be sealed in bags bearing the biohazard symbol and disposed of in a biohazard waste container. All transfers must be done in spill-proof containers. Individuals manipulating the vector will be required to wear personal protective equipment, such as gloves. Under no circumstances will the Investigator supply AVXS-101 to a third party, allow AVXS-101 to be used other than as directed by this clinical trial protocol, or dispose of AVXS-101 in any other manner.*

**J. Information on emergency response plans**

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

*In case of accidental spillage of AVXS-101 (e.g. on the workbench or on the floor), local procedures will be followed to contain and immediately disinfect the spill to prevent further spread. In case of accidental spillage of AVXS-101 (e.g. on the workbench or on the floor), local procedures will be followed to contain and immediately disinfect the spill to prevent further spread. To decontaminate areas affected (e.g. eradication of the GMOs), spillages in the operation room will be cleaned up with a bleach solution, in accord with NIH/CDC*

*guidance for handling of biosafety level 1 agents and the Pharmacy Manual. All contaminated materials will be disposed of locally by incineration or autoclaving. All other places will be cleaned, according to normal decontamination procedures (add reference to decontamination procedures at site).*

- 1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure.*
  - 2. Cover the spill with absorbent material. Starting at the edges and work towards the center.*
  - 3. Carefully pour disinfectant (bleach solution followed by alcohol wipes) over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.*
  - 4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes; viscous spills require 30 minutes.*
  - 5. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves*
  - 6. Discard absorbent material in Chemical waste bags.*
  - 7. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, and wipe with towels.*
  - 8. Discard all cleanup materials (soaked with disinfectant) in Chemical bag/ container, and any contaminated PPE in a biohazard bag. Close and secure the bags.*
  - 9. Place bag in a second biohazard bag, secure and dispose as per institutional guidelines for biohazardous waste.*
2. Methods for removal of the GMO(s) of the areas potentially affected  
*See 1 above; All materials used in the spill clean-up will be discarded as clinical waste, and will be incinerated.*
3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread  
*Not applicable (since exposure of plants or animals is not expected).*
4. Plans for protecting human health and the environment in the event of an undesirable effect  
*Institutional Review Board (IRB)/Independent Ethics Committee (IEC)-approved instructions should be provided to the patient's family and care giver(s) regarding use of protective gloves if/when they come into direct contact with the patient's bodily fluids and/or waste, as well as good hand-hygiene for a minimum of four weeks after gene replacement therapy. Additionally, patients are prohibited from donating blood for two years following the vector infusion.*