*ANNEX III A*

**INFORMATION REQUIRED IN NOTIFICATIONS CONCERNING RELEASES OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS**

I. GENERAL INFORMATION

A. Name and address of the notifier (company or institute)

*AveXis, Inc.*
*2275 Half Day Road, Suite 200*
*Bannockburn, IL 60015, United States*

B. Name, qualifications and experience of the responsible scientist(s)

*Brian Kaspar, PhD*
*Senior Vice President and Chief Scientific Officer, AveXis Inc.*

C. Title of the project

*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

*AVXS-101-CL-304: European - 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*

II. INFORMATION RELATING TO THE GMO

A. **Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s):**

1. scientific name;

*Adeno-associated Virus (AAV)*

2. taxonomy;

*Group: Group II (ssDNA)*
*Family: Parvoviridae*
*Genus: Dependovirus*
*Species: Adeno-associated virus*
*Serotype: 2 and 9*

3. other names (usual name, strain name, etc.);

*The parental virus concerned in this application is a primate (human) AAV. AAV is a parvovirus with a 4.7 KB single-stranded DNA genome; it is a small virus which infects humans and some other primate species, however it is not currently known to cause disease. AAV serotypes are defined by cell surface antigens. There are 12 serotypes as of 2009, and hundreds of isolates. A human isolate of AAV9 is used for AVXS-101. AAV9 is a unique serotype among the adeno-associated virus (AAV) family of viruses specifically recognized to target motor neurons which also has the ability to cross the blood-brain barrier. 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.*

4. phenotypic and genetic markers;

*Wild-type adeno-associated virus (AAV) is a single-stranded DNA virus with a non-enveloped icosahedral capsid with a diameter of approximately 25nm. The AAV genome is approximately 4.7 kilobase long and comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs), rep and cap. ORF 'rep' is composed of four overlapping genes encoding Rep proteins required for DNA replication, and ORF 'cap' contains overlapping nucleotide sequences coding for capsid proteins (VP1, VP2 and VP3) which interact together to form a capsid of an icosahedral symmetry. The inverted terminal repeats (ITRs) flank the two ORFs and contain all cis-acting functions required for DNA replication, packaging, integration into the host genome, and subsequent excision and rescue (Reviewed in Daya and Berns, 2008). There are several serotypes of adeno-associated virus and the serotype of AAV is determined by the capsid of the virion, which is integral to the tissue tropism and infection efficiency of AAV. The capsid proteins of AVXS-101 are derived from the AAV9 serotype. AVXS-101 contains the SMN cDNA expression cassette flanked by AAV2 inverted terminal repeat (ITR) sequences. The virus causes a very mild immune response at doses of exposure in the natural habitat of the human, lending further support to its apparent lack of pathogenicity. Gene therapy vectors using AAV can infect both dividing and quiescent cells, and persist in an extrachromosomal state without integrating into the genome of the host cell; AAV DNA predominantly remains as a non-integrated episome in the nucleus.*

5. degree of relatedness between donor and recipient or between parental organisms;

*The insert in vector construct comes from human SMN cDNA that is present in an expression cassette flanked by AAV2 inverted terminal repeat (ITR) sequences. The capsid proteins of AVXS-101 are derived from the AAV9 serotype. The overall level of amino acid identity in the capsid protein of AAV serotypes 1–9 is ~45% (Zincarelli et al., 2008). The variability between serotypes is not evenly distributed throughout the sequence of the capsid protein, but is concentrated in the looped-out domains that are displayed on the surface (Gao et a., 2003).*

6. description of identification and detection techniques;

*The presence of AAV may be detected in clinical samples in the following ways:*

(i) *Digital droplet PCR or ddPCR – The vector construct may be detected using the ddPCR assay (qPCR type assay) using primers and probe specific to the vector genome. The specificity of the ddPCR probes and primers permits its additional use as an identity test.*

(ii) *Enzyme-Linked ImmunoSpot (Elispot) – A T-cell response to AAV may be detected via blood.*

(iii) *Enzyme-Linked Immunosorbent Assay (ELISA)- AAV antibodies may be detected via ELISA from serum*

7. sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;

*The sensitivity/ specificity of the three methods for detection of wild type AAV is not apparent in the scientific literature, likely due to a lack of clinical utility of diagnostic methods due to the apparent lack of pathogenicity of the virus. It is possible to estimate sensitivity of detection of wild type AAV based on what is known regarding recombinant AAV vectors; However, it must be noted that such sensitivities are achieved in samples of purified vector, with essentially no interference from the sample matrix. Any clinical sample will be subject to tissue sample interference, which must be assessed and may reduce sensitivity. ddPCR will be used to detect AVXS-101 shortly after dosing and for the weeks immediately following administration. It is during this time that we would expect the highest levels of shed AVXS-101 to be present and monitoring this time will give an estimation of the total amount of AVXS-101 shed from the patient. This method has a limit of detection estimated to be ~ 1.10E+05 GC/mL of undiluted urine or saliva or ~ 1.1E+06 GC/g of stool.*

8. description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts;

*The human adeno-associated virus (AAV) was discovered in 1965 as a contaminant of adenovirus (Ad) preparations. It is a globally endemic infection of humans, as demonstrated by the cross-reactivity of antibodies in the population to one or more AAV serotypes. There are no known natural predators, preys, parasites, competitors or symbionts associated with AAV, although it does require helper functions of co-infecting viruses.*

9. organisms with which transfer of genetic material is known to occur under natural conditions;

*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. Evolution of AAV viruses (like all viruses) is directed by spontaneous mutation or homologous recombination with other viruses of the same species, where such genetic modification confers a selective advantage. Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus which is permissive in that species.*

10. verification of the genetic stability of the organisms and factors affecting it;

*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.*

11. pathological, ecological and physiological traits:

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

*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'. All of the DNA from the wild-type AAV9 has been removed and replaced with the genes described below (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. 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.*

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

*Wild type AAV enters cells by interaction of specific viral capsid epitopes with cell surface receptors, influencing the infection efficiency, host range and specific tissue tropism of a virus. Once internalized, the virus encounters a weakly acidic environment which is sufficient to allow penetration into the cytosol. AAV rapidly moves to the cell nucleus and accumulates perinuclearly beginning within 30 min after the onset of endocytosis. Escape of AAV from the endosome and trafficking of viral particles to the nucleus are unaffected by the presence of adenovirus. Within 2h, viral particles could be detected within the cell nucleus, suggesting that AAV enters the nucleus prior to uncoating. The majority of the intracellular virus particles remain in a stable perinuclear compartment and slowly*

*penetrate into the nucleus, possibly through the nuclear pore complex (Bartlett et al., 2000).*

*After entry into the host cell nucleus, wild type AAV can follow either one of two distinct and interchangeable pathways of its life cycle: the lytic or the lysogenic. The former develops in cells infected with a helper virus whereas the latter is established in host cells in the absence of a helper virus. During this period, AAV undergoes productive infection characterized by genome replication, viral gene expression, and virion production (reviewed in Daya and Berns, 2008). When wild type AAV infects a human cell alone, its gene expression program is autorepressed and latency ensues by preferential integration of the virus genome into a region of roughly 2-kb on the long arm (19q13.3-qter) of human chromosome 19. 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 and following naturally acquired infection, wild type AAV DNA mostly persists as circular double stranded episomes in human tissues.*

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

*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. AAV mostly survives in the environment as a persistent infection in the host. Replication of 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 helper 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. AAV is not known to form survival structures but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization (Tenenbaum et al., 2003).*

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

*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.*
*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.*

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

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

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

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

12. Nature of indigenous vectors:

(a) sequence;

*The genomic DNA of AAV is approximately 4.7 kilobase long and comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs), rep and cap. ORF 'rep' is composed of four overlapping genes encoding Rep proteins required for DNA replication, and ORF 'cap' contains overlapping nucleotide sequences coding for capsid proteins (VP1, VP2 and VP3) which interact together to form a capsid of an icosahedral symmetry. The inverted terminal repeats (ITRs) flank the two ORFs and contain all cis-acting functions required for DNA replication, packaging, integration into the host genome, and subsequent excision and rescue (Reviewed in Daya and Berns, 2008). There are several serotypes of adeno-associated virus and the serotype of AAV is determined by the capsid of the virion, which is integral to the tissue tropism and infection efficiency of AAV.*

(b) frequency of mobilisation;

*The presence of natural mobile genetic elements such as transposons or plasmids related to AAV has not been reported.*

(c) specificity;

*AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV in vitro, provided it is in the presence of a helper virus to which that species is permissive It is not known* whether zoonosis occurs in nature. *The wild type virus does not contain any gene that confers resistance to known antibiotics.*

13. History of previous genetic modifications.

*From an evolutionary history point of view, genetic variations of the ancestral AAV resulted in various viral lineages and serotypes. The overall level of amino acid identity in the capsid protein of AAV serotypes 1–9 is ~45% (Zincarelli et al., 2008). The variability between serotypes is not evenly distributed throughout the sequence of the capsid protein, but is concentrated in the looped-out domains that are displayed on the surface (Gao et a., 2003).*

B. **Characteristics of the vector:**

1. nature and source of the vector;

*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-β-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.*

2. sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO;

*One of the two adeno-associated vector (AAV) inverted terminal repeats (ITRs) has been modified to promote intramolecular annealing of the transgene, thus forming a double-stranded transgene ready for transcription. The vector construct is illustrated in (Figure 1).*

**Figure 1: Vector Construct**


3. frequency of mobilisation of inserted vector and/or genetic transfer capabilities and methods of determination;

*The vector construct contains the SMN 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. AVXS-101 is unable to replicate independently, even in the presence of a helper virus, since it lacks the rep and cap genes required for rescue/packaging.*

4. information on the degree to which the vector is limited to the DNA required to perform the intended function.

*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. **Characteristics of the modified organism:**

1. Information relating to the genetic modification:

(a) methods used for the modification;

*The plasmids used in the manufacture of AVXS-101 were constructed using standard molecular biological techniques for the precise excision and ligation of component elements using specific restriction enzymes followed by transduction and amplification in bacterial cells at each stage. The desired product plasmid was selected at each stage using antibiotic resistance marker genes. The final plasmid stocks used in the manufacture of AVX-101 carry the gene for resistance to kanamycin for selection purposes, and have been amplified and tested to confirm the correct DNA sequence.*

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

*AVXS-101 is produced by co-transfecting a Master Cell Bank (MCB) of Human Embryo Kidney (HEK) 293 cells with the three plasmid stocks:*

*1) pSMN contains the SMN cDNA expression cassette flanked by AAV2 inverted terminal repeat (ITR) sequences;*

*2) pAAV plasmid encodes the 4 wild-type AAV rep proteins from serotype 2 and the 3 wild-type AAV VP capsid proteins from serotype 9;*

*3) pHELP Adenovirus Helper Plasmid required for AAV replication in HEK293 cells. Note that the required helper functions are provided as a plasmid, and not a viable adenovirus.*

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

*Plasmids were subjected to restriction enzyme digestion and were confirmed by DNA plasmid sequencing. For the region in which sequence data was obtained, the sequenced sample was 100% identical to the expected sequence.*

(e) methods and criteria used for selection;

*Antibiotics are not used in the manufacturing process used to construct the AVXS-101vector in HEK293 cells from the component plasmids. Each lot of AVSX-101 is tested for identity, purity (including residual plasmid DNA) and freedom from adventitious agents (including replication competent AAV) and must meet specified acceptance criteria prior to use.*

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

*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.*

*The DNA sequence for the AVXS-101 plasmid is described in Table 1*

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**Table 1: Description of the AVXS-101 Plasmid DNA Sequence**

| 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-β-Actin Hybrid (CB) Promoter | Portion of the CMV immediate/early enhancer<br>Chicken-β- 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 *cis* for both viral DNA replication and packaging of the rAAV vector genome |

2. Information on the final GMO:

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

*AVXS-101 is a non-replicating recombinant AAV9 containing the human survival motor neuron (SMN) gene under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB). The expression cassette will be transcribed and translated by host cell enzymes leading to expression of SMN. The original AAV genome has been extensively modified. It no longer carries any of the original viral genes and has had all extraneous viral nucleic acids removed. 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. AVXS-101 outer capsid is derived from AAV9.*

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

*The original AAV genome has been extensively modified. It no longer carries any of the original viral genes and has had all extraneous viral nucleic acids removed. 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.*

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

*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.*

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

*Dosing and efficacy of scAAV9-SMN was evaluated in SMNΔ7 mice, non-human primates, and pigs using systemic delivery or directly to the cerebral spinal fluid (CSF) via single injection. Widespread transgene expression was observed throughout the spinal cord in mice nonhuman primates and pigs. (Foust 2009, Bevan 2011, Meyer 2015)*

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

*AVXS-101 codes for the human Survival motor neuron (SMN) protein, the loss of which is be the root cause of SMA. SMN, in collaboration with partner proteins, functions in the assembly of small nuclear ribonucleoproteins (snRNPs)and pre-mRNA splicing which is important for the survival and functioning of the spinal motor neurons (Burghes, 2009).*

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

*Droplet digital PCR or ddPCR with primers and probe specific to SMN gene in AVXS-101 can be used to identify and detect AVXS-101.*

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

*Droplet digital PCR or ddPCR uses specific primers and probe to SMN gene. It has a limit of detection estimated to be ~ 1.10E+05 GC/mL of undiluted urine or saliva or ~ 1.1E+06 GC/g of stool.*

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

*A Phase I clinical trial for AVXS-101 (AVXS-101-CL-101) is currently ongoing in the USA. As of 7 August 2017 , AVXS-101 appears to have a favorable safety profile and appears to be generally well-tolerated in patients studied to date in the AVXS-101-CL-101 study. Fifteen SMA Type 1 patients were enrolled and received a one-time intravenous infusion of AVXS-101. Patients are followed for safety for two years; the last patient was enrolled Dec 2015.*

*A Phase 3 clinical trial for AVXS-101 (AVXS-101-CL-303) was initiated in the USA in September 2017. The open-label, single-arm, single-dose, multi-center trial – known as STR1VE – is designed to evaluate the efficacy and safety of a one-time IV infusion of AVXS-101 of 1.1 x $10^{14}$ vg/kg, which is equivalent to the proposed therapeutic dose received by the second dosing cohort in the Phase 1 trial, in patients with SMA Type 1. The trial will enroll a minimum of 15 patients with SMA Type 1 who are less than six months of age at the time of gene therapy, and who have one or two copies of the SMN2 backup gene as determined by genetic testing and bi-allelic SMN1 gene deletion or point mutations.*

*A Phase 1 clinical trial for AVXS-101 (AVXS-101-CL-102) was initiated in the USA in December 2017. The open-label, dose-comparison, multi-center Phase 1 trial – known as STRONG – is designed to evaluate the safety, optimal dosing, and proof of concept for efficacy of AVXS-101 in two distinct age groups of patients with SMA Type 2, utilizing a one-time IT route of administration. The trial will enroll 27 infants and children with a genetic diagnosis consistent with SMA, including the bi-allelic deletion of SMN1 and three copies of SMN2 without the SMN2 genetic modifier, who are able to sit but have no historical or current ability to stand or walk.*

*Two dosage strengths will be evaluated and patients will be stratified into two age groups: patients less than 24 months, and patients at least 24 months but less than 60 months. There will be at least a four-week interval between the dosing of the first three patients for each dose being studied and, based on the available safety data, a decision will be made whether to proceed.*

- *Cohort 1 (Dose A) will receive a dose of 6.0 x $10^{13}$ vg of AVXS-101 and enroll three patients less than 24 months of age.*
    - *If safety is established according to the Data Safety Monitoring Board (DSMB), the study will proceed to Cohort 2.*
- *Cohort 2 (Dose B) will receive a dose of 1.2 X $10^{14}$ vg of AVXS-101 and enroll three patients less than 60 months of age.*
    - *If safety is established according to the DSMB, an additional 21 patients will be enrolled until there are a total of 12 patients less than 24 months, and 12 patients at least 24 months but less than 60 months of age, who have received Dose B.*

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

*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. 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.*

(i) toxic or allergenic effects of the GMOs and/or their metabolic products;

*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) AAV2 and AAV9 are non-pathogenic, toxigenic, virulent, allergenic or a carrier (vector) of a pathogen. Based on data form the Phase 1 study currently available through 07 August 2017, AVXS-101 appears to be safe and well tolerated when administered to infants with SMA, and has demonstrated encouraging early evidence of clinically meaningful efficacy in this otherwise devastating neurodegenerative disease.*

*AVXS-101 administration in the AVXS-101-CL-101 study has resulted in marked and positive impact on motor function and motor milestone achievement. As of 20 Jan 2017, mean increases from baseline in CHOP-INTEND scores of 7.7 points in Cohort 1 and 24.7 points in Cohort 2 were observed, reflecting a substantial improvement in motor function relative to the natural history of Type 1 SMA. All patients in both dosing cohorts experienced a sustained increase in CHOP-INTEND scores above baseline; in Cohort 2, 11 of 12 patients achieved CHOP-INTEND scores greater than or equal to 40. Many patients in Cohort 2 have achieved and sustained motor milestones (such as sitting unassisted) which are not achieved in the natural history of Type 1 SMA as described in recent published natural history studies. Given the devastating clinical course of Type 1 SMA, the irreversible and progressive nature of motor neuron loss as the disease progresses, and the urgent and substantial unmet medical need in this serious disorder, the available data strongly support a positive benefit/risk relationship and strongly support continued study of AVXS-101 in patients with SMA.*

*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.*

*Because of the limited number of patients treated with AVXS-101 to date, the potential risks associated with AVXS-101 are not fully known at this time. Patients could develop an immune response to the AAV9 viral vector, which could interfere with or prevent future use of gene transfer interventions using this vector. Elevated liver function tests have been observed in the ongoing AVXS-101-CL-101 trial, which is believed to reflect a T-cell immune response to the AAV9 vector. None of the liver enzyme abnormalities observed in the trial were accompanied by clinical sequelae, and all have resolved following treatment with prednisolone. Although no other treatment-related AEs have been reported to date, other potential risks of treatment may exist that are not currently known given the limited clinical experience to date, and the benefit/risk profile will continue to become better characterized with continued study.*

*Nonclinical data in nonhuman primates and mouse models of SMA provide additional support for a positive benefit/risk relationship, and support continued clinical investigation of AVXS-101 in patients with SMA. Efficacy studies in the SMNΔ7 mouse model of SMA have demonstrated significant apparent benefits in several disease-associated phenotypes, including motor functioning, body weight, and survival. Local vascular necrosis of the ear pinna was observed in some treated SMNΔ7 mice. Because similar findings have been reported in other studies of SMNΔ7 mice and have been observed in several other SMA mouse models, it is believed this finding is unlikely to be related to treatment. In preclinical toxicology studies conducted in wild-type mice and cynomolgus macaques, no toxicologically significant treatment related effects were seen on body weight or on hematology, clinical chemistry and histopathology evaluations.*

*Taken together, results from the clinical and nonclinical studies to date support continued clinical investigation of the efficacy and safety of AVXS-101 in patients with SMA Type 1, and additionally support further investigation of intravenous and intrathecal administration of AVXS-101 in a broader population of patients with SMA.*

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

*Neither wild type AAV nor the experimental vector AVXS-101 is known to be pathogenic to humans. 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-β-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.*

(iii) capacity for colonisation;

*AVXS-101 does not contain any of the viral genes necessary for replication (rep, cap) and thus is replication defective even in the presence of a helper virus. Only in the hypothetical situation that a cell is co-infected with AVXS-101, wild type AAV, and helper virus, replication of (disseminated) AVXS-101 could occur. Thus, the pathogenicity of AVXS-101 is expected to be even less than that of its parental AAV2 or AAV9 viruses, which are already considered non-pathogenic.*
*The effects of unintended exposure of human beings to AVXS-101 are the same as those from intended exposure to subjects (patients): effects related to the expression SMN protein, induction of anti-AAV9 immune responses. The likelihood that these effects occur and/or cause harmful effects are negligible, because unintended exposure of human beings to (infectious) AVXS-101 can only be many orders of magnitude lower than the subjects' exposure due to the replications incompetence of AVXS-101 and the limited amount and duration (if any) of infections AVXS-101 shedding from subjects.*

(iv) if the organism is pathogenic to humans who are immunocompetent:

- diseases caused and mechanism of pathogenicity including invasiveness and virulence,

- communicability,

- infective dose,

- host range, possibility of alteration,

- possibility of survival outside of human host,

- presence of vectors or means of dissemination,

- biological stability,

- antibiotic-resistance patterns,

- allergenicity,

- availability of appropriate therapies.

*Neither wild type AAV nor the experimental vector AVXS-101 is known to be pathogenic to humans. The administration of an AAV vector has the risk of causing immune-mediated hepatitis. For patients who have positive serology for hepatitis B or C, administration of AAV vector may represent an unreasonable risk; therefore, negative serology testing must be confirmed prior to treatment.*

(v) other product hazards

*None known or anticipated.*

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

A. **Information on the release:**

1. description of the proposed deliberate release, including the purpose(s) and foreseen products;

*Following the approval of the Clinical Trial Authorization for AVXS-101, it is planned to make the product available to clinical trial site(s)for use in accordance with a Clinical Trial Protocol entitled: 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.*

*2. foreseen dates of the release and time planning of the experiment including frequency and duration of releases;*

*The use of AVXS-101 will commence following approval of the Clinical Trial Authorization. It is anticipated that the trial will start in April 2018 and will end June 2023.*

3. preparation of the site previous to the release;

*No specific preparation of sites will be required prior to release, unless local procedures dictate otherwise. Information on handling will be available to medical professionals involved in product preparation via the Clinical Trial Protocol and Pharmacy and Handling Instructions. The Sponsor will provide pharmacy and handling training to those personnel involved in the clinical trial. Dose preparation is to be performed under sterile conditions, a BioSafety Cabinet (BSC) is recommended for sterility, but not required.*

4. size of the site;

*Up to approx. 2-3 patients are expected to be enrolled at each site.*

5. method(s) to be used for the release;

*Patients will receive a one-time dose of AVXS-101 via intravenous (IV) infusion. AVXS-101 drug product is supplied in clear, single-use, sterile vials. The clinical site pharmacist will prepare the AVXS-101 product under sterile conditions. The total vector genome (vg) dose will be calculated based on patient's body weight. The appropriate number and size of vials will be determined for each patient based on body weight. All transfers of AVXS-101 must be done in spill-proof containers. Individuals manipulating the vector will be required to wear personal protective equipment. The empty vial and syringes used for delivery of the vector should be sealed in bags bearing the biohazard symbol and returned to AveXis unless not allowed per the research site's SOP.*

*The AVXS-101 intravenous infusion procedure should be performed under sterile conditions in a PICU patient room or other appropriate setting (e.g., interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management. AVXS-101 will be delivered one-time through a venous catheter inserted into a peripheral limb vein (arm or leg) and slowly infused over approximately 30 minutes.*

*Following administration, patients should return to an appropriate designated post-operative or pediatric intensive care unit to ensure close monitoring of vital signs and adverse reactions. Vitals should be monitored every 15 minutes for four hours and every hour for 24 hours. Patients may be discharged 24 hours after the infusion, based on Investigator judgment.*

*Blood and urine samples will be collected at scheduled visits. Internal transport of the samples within the investigational site should be done in spill-proof containers. Samples for laboratory tests required during the in-patient vector infusion period prior to dosing will be collected and processed by the investigative site's local laboratory.*

6. quantities of GMOs to be released;

*The AVXS-101 intravenous infusion procedure should be performed under sterile conditions in a PICU patient room or other appropriate setting (e.g., interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management. AVXS-101 will be delivered one-time through a venous catheter inserted into a peripheral limb vein (arm or leg) and will be administered with a syringe pump, slowly infused over approximately 30 minutes. The total vector genome (vg) dose will be calculated based on patient's body weight. Patients will receive a one-time dose of AVXS-101 at 1.1 X $10^{14}$ 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. At least fifteen (15) patients with 2 copies of SMN2, at least twelve (12) patients with 3 copies of SMN2 and at least seventeen (17) patients with 4 copies of SMN2 that are ≤6 weeks of age at the time of gene replacement therapy (Day 1) will be enrolled and treated with single dose of AVXS-101.*

7. disturbance on the site (type and method of cultivation, mining, irrigation, or other activities);

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

8. worker protection measures taken during the release;

*Safety measures for biosafety level 1 agents will be utilized. Preparation AVXS-101 should be completed in accord with local/national aseptic techniques. The clinical site pharmacist will prepare the AVXS-101 vector product under sterile conditions. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory.*

*BSL-1 workers should: Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.*

9. post-release treatment of the site;

*Post-release treatment of the site will not be necessary, provided the precautions outlined in the Pharmacy Instructions and Handling Instructions in the AVXS-101-CL-304 Pharmacy and Dose Administration Manual are adhered to when preparing or administering the product or when dealing with accidental spillages and breakages.*

10. techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment;

*All materials used for injection, including sterile drapes, needles, and syringes in contact with the vector must be sealed in leak-proof primary and secondary containers. All waste must be double bagged in bags bearing the biohazard symbol. The bag must then be 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. The empty vial(s) and syringe used for delivery of the vector should be double bagged in bags bearing the biohazard symbol and sealed, then returned to AveXis.*

11. information on, and results of, previous releases of the GMOs, especially at different scales and in different ecosystems.

*In an ongoing Phase 1 trial, safety of AVXS-101 was assessed in SMA Type 1 patients via intravenous infusion in the AVXS-101-CL-101 clinical study. Fifteen SMA Type 1 patients were enrolled and received a one-time intravenous infusion of AVXS-101. Patients are followed for safety for two years; the last patient was enrolled Dec 2015. At the end of Phase1, patients are encouraged to enroll in a long-term follow up study (AVXS-101 LT-001).*

*As of 20 January 2017, AVXS-101 appears to have a favorable safety profile and to be generally well-tolerated; treated patients have demonstrated improvement in motor function and achievement of developmental milestones. Additionally, patients treated to-date with AVXS-101 have demonstrated improved nutritional status including lower instances of required feeding support (e.g., G-tube, NJ tube) due to the inability to swallow when compared with untreated patients. 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.*

B. **Information on the environment (both on the site and in the wider environment):**

1. geographical location and grid reference of the site(s) (in case of notifications under Part C the site(s) of release will be the foreseen areas of use of the product);

*Following the approval of the Clinical Trial Authorization for AVXS-101, it is planned to make the product available to the following clinical trial site for use in accordance with the Clinical Trial Protocol [Protocol # AVXS-101-CL-302 and AVXS-101-CL-304]:*

***AVXS-101-CL-302***

Centre Hospitalier Régional Hôpital La Citadelle
Neuropédiatrie - Centre de Référence des Maladies Neuromusculaires
Boulevard du 12ième de Ligne 1
4000 Liège
Belgium
Contact: Aurore Daron (T: +32 4 321 56 00 or CRMN +32 4 321 83 15)

UZ Gent
Neuromuscular reference center
Heymanslaan 10
9000 Gent
Belgium
Contact: Nicolas Deconinck (nicolas.deconinck@uzgent.be)

***AVXS-101-CL-304***

Centre Hospitalier Régional Hôpital La Citadelle

Boulevard du 12ième de Ligne 1
4000 Liège
Belgium
Contact: Dr Servais Laurent (e-mail: l.servais@institut-myologie.org - T: +3243218315)

2. physical or biological proximity to humans and other significant biota;

*AVXS-101 will be administered to the patient by a medical professional in a medical facility. The product itself will be stored prior to administration in a secure environment (pharmacy).*

*Given the nature of the product administration (intravenous), and the transient/ low levels of shedding expected, the risk of unintended exposure to AVXS-101 to humans and other biota is minimal.*

3. proximity to significant biotopes, protected areas, or drinking water supplies;

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

4. climatic characteristics of the region(s) likely to be affected;

*The clinical trial of AVXS-101 will occur in Australia, Belgium, Canada, Germany, Israel, Japan, Spain, Taiwan, United Kingdom, and United States. The risk of release of AVXS-101 into the environment is unrelated to climatic characteristics. The stability of AVXS-101 in the environment is unchanged from that of wild-type AAV.*

5. geographical, geological and pedological characteristics;

*The risk of release of AVXS-101 into the environment is unrelated to these characteristics. The stability of AVXS-101 in the environment is unchanged from that of wild-type AAV.*

6. flora and fauna, including crops, livestock and migratory species;

*Given the nature of the product administration (intravenous) and the transient/ low levels of shedding expected, the risk of unintended exposure of flora and fauna to AVXS-101 is minimal.*

7. description of target and non-target ecosystems likely to be affected;

*No ecosystems are targeted in the use of AVXS-101. Ecosystems are not expected to be affected.*

8. a comparison of the natural habitat of the recipient organism with the proposed site(s) of release;

*The natural habitat of wild type AAV2/9 is primates (human). AVXS-101 will be administered to human beings, though the genetic construct is deleted for the rep and cap genes, rendering it unable to replicate, even in the presence of a helper virus.*

9. any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

*None known.*

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

A. **Characteristics affecting survival, multiplication and dissemination:**

1. biological features which affect survival, multiplication and dispersal;

*AVXS-101 is non-pathogenic wild-type AAV2/9, modified by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus. As a derivative of primate (human) AAV2/9, the primary indigenous vector of AVXS-101 is human beings.*

*AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV in vitro, provided it is in the presence of a helper virus to which that species is permissive. It is not known whether zoonosis occurs in nature, nor whether other species can act as carriers or vectors under natural conditions*

*The survival of AVXS-101 outside of the host is expected to be the same as wild-type AAV.*

2. known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.);

*Environmental conditions which may affect survival AVSX-101 outside the host are temperature, pH and environmental humidity. AVXS-101 is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus.*

3. sensitivity to specific agents.

*The genetic modifications made during the construction of AVXS-101 from wild type AAV2/9 are not expected to affect its sensitivity to physical and chemical inactivation.*

*Physical inactivation:*

*Wild-type AAV virus is inactivated outside the host by exposure to pH > 9 and by autoclaving for 30-45 minutes at 121°C.*

B. **Interactions with the environment:**

1. predicted habitat of the GMOs;

*The predicted habitat of AVXS-101 is humans where it is expected to persist as episomal concatemer structures that are transcriptionally active. AVXS-101 is a non-replicative version of a non-pathogenic wild-type AAV, modified by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus.*

2. studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses;

*AVXS-101 is a replication-incompetent virus derived from AAV2/9. The genetic modifications do not affect its natural host and tissue tropism. No specific studies have been conducted regarding transmission of AVXS-101 between humans or animals Studies have shown that some vector can be excreted from the body for up to a few weeks after injection/infusion; this is called "viral shedding". 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, because the vector is non-pathogenic and cannot replicate, it is believed that shed vector is unlikely to result in clinically significant adverse effects. 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 four weeks after the injection.*

3. genetic transfer capability:

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

*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.*

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

*AVXS-101 is a replication-incompetent virus derived from AAV2/9. The genetic modifications do not affect its natural host and tissue tropism. No transfer of genetic material between the GMO and other organisms is predicted. 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.*

*This scenario is expected to be a rare event, and would only result in the production of more wild type AAV and more AVXS-101 vector particles (which would still lack rep and cap genes and consequently could not be self-sustaining).*

4. likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism;

*The selective pressure on AVXS-101 will be towards reversion to wild-type, since both gene deletions (rep and cap) are required for rescue and replication of the organism in its host species. The likelihood of this reversion is considered low, since it would require genetic exchange by homologous recombination with wild-type AAV which could only occur if human cells were simultaneously infected with both wild type AAV, AVXS-101 and a helper virus (e.g. adenovirus). The transgene (hSMN) is not expected to confer any advantage to the GMO in terms of survival and selective pressure.*

5. measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability;

*AVXS-101 is unable to replicate independently, even in the presence of a helper virus, since it lacks the rep and cap genes required for rescue/packaging. Therefore, infection leading to replication of the GMO (and therefore potential for dispersal) is not possible under normal circumstances. AVXS-101 is expected to be genetically stable. In general, DNA viruses have greater genetic stability than RNA viruses. Evolution of AAV viruses (like all viruses) is directed by spontaneous mutation or homologous recombination with other viruses of the same species, where such genetic modification confers a selective advantage. Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus which is permissive in that species (triple-infection). In the case of AVXS-101, such recombination could only result in the exchange of the hSMN expression cassette with the rep and cap genes of the wild type virus. It is not possible for the AAV genome to contain both rep/cap genes and the transgene, as this is beyond the packaging limit of the virion. Therefore, the only mechanism by which the transgene could be mobilized is through a triple infection of the same cell by AVXS-101 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, and would only result in the production of more wild type AAV and more AVXS-101 vector particles (which would still lack rep and cap genes and consequently could not be self-sustaining).*

6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.;

*Outside of the host, non-lipid enveloped viruses such as AAV are resistant to low level disinfectants, survive outside of the laboratory environment and can be transmitted via fomites. AAV does not form survival structures but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization. Dispersal (dissemination) of AAV is not documented definitively, but is likely through inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion. AVXS-101 is a replication-incompetent virus derived from AAV2/9. The genetic modifications do not affect its survival outside the host or probable mode of dissemination. However, the lack of replicative ability prevents multiplication and therefore severely limits its ability to disseminate.*

7. description of ecosystems to which the GMOs could be disseminated.

*Dissemination of AVXS-101 would most likely only occur between human beings, since it is derived from AAV2/9. However, no replication is expected in normal cells of treated individuals exposed to the replication-deficient virus, or from exposure of uninfected people to treated individuals.*

8. potential for excessive population increase in the environment;

*Dissemination of AVXS-101 would most likely only occur between human beings, since it is derived from AAV2/9. However, no replication is expected in normal cells of treated individuals exposed to the replication-deficient virus, or from exposure of uninfected people to treated individuals.*

9. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s);

*AVXS-101 is a replication-incompetent virus derived from AAV2/9 and is therefore at a competitive disadvantage when compared to its parent strain / wild type AAV. The transgene (human survival motor neuron) is not expected to confer any advantage to the GMO in terms of survival and selective pressure.*

10. identification and description of the target organisms if applicable;

*AVXS-101 is being developed for the treatment of pediatric patients diagnosed with spinal muscular atrophy (SMA).*

11. anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable;

*In treated subjects, AVXS-101 is expected to target the motor neurons in the CNS and also target key systemic tissues. Following treatment, the vector is expected to persist for months or years, primarily as stable episomal structures in the cells that are transcriptionally active*

*The presence of the hSMN gene under the transcriptional control under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB) is expected to result in expression of functional hSMN. Increased levels of functional SMN protein in the target cells are expected to arrest or reverse motor neuron cell death and denervation of skeletal muscle.*

12. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction;

*AVXS-101 is derived from the non-pathogenic AAV2/9. AVXS-101 is non-replicative by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus. Therefore, infection leading to replication of the GMO (and therefore potential for dispersal) is not possible under normal circumstances. AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV in vitro, provided it is in the presence of a helper virus to which that species is permissive. It is not known whether zoonosis occurs in nature, nor whether other species can act as carriers or vectors under natural conditions. However, given the inability to replicate and site of administration, the possibility of exposure of AVXS-101 to non-humans is considered negligible.*

*The non-target organisms which could conceivably be affected are unintended human recipients (healthcare workers and close contacts of the patient). It is not expected that transmission would lead to adverse effects in healthy humans since neither wild type AAV nor AVXS-101 are known to be pathogenic. In the unlikely event that transmission to a healthy unintended human recipient occurs it is likely that the safety profile in healthy subjects would be at worst similar to those expected in patients.*

13. likelihood of post-release shifts in biological interactions or in host range;

*The likelihood of post-release shifts in biological interactions or host range is negligible. AAV enters cells by interaction of specific viral capsid epitopes with cell surface receptors. The inserted gene in AVXS-101 is hSMN that is packaged in viral capsid proteins derived from AAV9, and therefore would not be expected to alter the host range or cell tropism of the virus. The gene deletions in AVXS-101 prevent the ability of the virus to replicate independently, but do not affect the packaging viral capsid proteins so would not be expected to have any effect on host range or cell tropism. In summary, the host range and tropism of AVXS-101 is expected to be identical to wild type AAV.*

14. known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens;

*None known or predicted.*

15. known or predicted involvement in biogeochemical processes;

*None known or predicted.*

16. other potential interactions with the environment.

*None known or predicted.*

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

A. **Monitoring techniques:**

1. methods for tracing the GMOs, and for monitoring their effects;

*Following administration of gene replacement therapy, patients should remain in the dosing suite or be moved to an appropriate designated setting to ensure close monitoring of vital signs and adverse events for a minimum of 24 hours after the start of gene replacement therapy. Patients may be discharged 24 hours after the infusion, based on Investigator judgment.*

*Studies have shown that some vector can be excreted from the body for up to a few weeks after injection/infusion; this is called "viral shedding". 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, because the vector is non-pathogenic and cannot replicate, it is believed that shed vector is unlikely to result in clinically significant adverse effects. 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 four weeks after the injection. Additionally, patients are prohibited from donating blood for two years following the vector injection.*

*Monitoring is not planned or considered neccessary for CL-304 study.*

2. specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques;

*The detection and identification of AVXS-101 can be done using digital droplet PCR (ddPCR) using specific primers and probe against the hSMN gene that is absent from the wild-type AAV. No monitoring is planned or considered neccessary for CL-304 study.*

3. techniques for detecting transfer of the donated genetic material to other organisms;

*No monitoring of unintended recipients is planned or considered necessary.*

4. duration and frequency of the monitoring.

*Not applicable.*

B. **Control of the release:**

1. methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of release or the designated area for use;

*Patients will receive a one-time dose of AVXS-101 via intravenous (IV) infusion. A pharmacist will prepare the AVXS-101 vector product under sterile conditions.*

*AVXS-101 for intravenous infusion will be supplied pre-mixed with saline in crystal zenith vials. The appropriate number and size of vials will be determined for each patient based on body weight. The total vector genome (vg) dose will be calculated based on patient's body weight. Immediately prior to dosing, AVXS-101 will be transferred from the drug product vials to an appropriately sized polypropylene dosing syringe. All transfers must be done in spill-proof containers. Individuals manipulating the vector will be required to wear personal protective equipment. The empty vial used for delivery of the vector should be sealed in bags bearing the biohazard symbol and then returned to AveXis.*

*The AVXS-101 intravenous infusion procedure should be performed under sterile conditions in a PICU patient room or other appropriate setting (e.g., interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management. AVXS-101 will be delivered one-time through a venous catheter inserted into a peripheral limb vein (arm or leg) and will be slowly infused over approximately 30 minutes.*

*Following administration of gene replacement therapy, patients should remain in the dosing suite or be moved to an appropriate designated setting to ensure close monitoring of vital signs and adverse events for a minimum of 24 hours after the start of gene replacement therapy. Patients may be discharged 24 hours after the infusion, based on Investigator judgment.*

*Blood and urine samples will be collected at scheduled. Internal transport of the samples within the investigational site should be done in spill-proof containers. Samples for laboratory tests required during the in-patient vector infusion period prior to dosing will be collected and processed by the investigative site's local laboratory.*

2. methods and procedures to protect the site from intrusion by unauthorised individuals;

*AVXS-101 will be administered to the patient by a medical professional in a medical facility. The product itself will be stored prior to administration in a secure environment (pharmacy). Intrusion by unauthorized individuals is therefore considered adequately controlled.*

3. methods and procedures to prevent other organisms from entering the site.

*No other procedures are considered necessary to prevent other organisms from entering the site, since AVXS-101 is a replication-incompetent version of wild-type AAV2/9. Within a medical facility, general pest control and cleaning procedures will be in place as dictated by site specific procedures for general hygiene.*

C. **Waste treatment:**

1. type of waste generated;

*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.*

2. expected amount of waste;

*Patients will receive a one-time dose of AVXS-101 equivalent to the dose received by the second dosing cohort in the Phase 1 study (AVXS-101-CL-101) administered via IV infusion. All materials used for the on-time injection including flush syringes, dose syringes, infusion tubing, needle used for preparing the dose, syringe cap, alcohol wipes and drapes would should be double bagged in bags bearing the biohazard symbol and sealed.*

3. description of treatment envisaged.

*All waste must be double bagged in bags bearing the biohazard symbol and sealed. The bag must then be 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. The empty vial(s) used for delivery of the vector should be double bagged in bags bearing the biohazard symbol and sealed, then returned to AveXis.*

D. **Emergency response plans:**

1. methods and procedures for controlling the GMOs 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. To decontaminate areas affected (e.g. eradication of the GMOs), spillages in the operation room will be cleaned up as indicated below:*

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 decontamination of the areas affected, e.g. eradication of the GMOs;

*There are no other specific procedures required or planned for decontaminating areas in the case of unexpected spread, since the risk of spread is considered negligible. In the unlikely event that transmission to an unintended human recipient occurred, this would likely be a local occurrence affecting a healthcare professional or close contact of a treated individual.*

3. methods for disposal or sanitation of plants, animals, soils, etc. that were exposed during or after the spread;

*The predicted habitat of AVXS-101 is humans where it is expected to persist as episomal concatemer structures that are transcriptionally active. AVXS-101 is a non-replicative version of a non-pathogenic wild-type AAV, modified by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus. Decontamination of plants, (non-human) animals and soils will not be required.*

4. methods for the isolation of the area affected by the spread;

*There are no specific plans for isolation of an area since the spread of AVXS-101 is highly unlikely.*

5. plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

*Following administration of gene replacement therapy, patients should remain in the dosing suite or be moved to an appropriate designated setting to ensure close monitoring of vital signs and adverse events for a minimum of 24 hours after the start of gene replacement therapy. Patients may be discharged 24 hours after the infusion, based on Investigator judgment. Information will be collected regarding all individual adverse events and submitted to competent authorities as required for a Serious Unexpected Suspected Adverse Reaction (SUSAR). Development Safety Update Reports will also be submitted as required while the trial is active.*

*Subsequent to completion of the study, patients will be asked to roll over into the long-term follow-up study.*