AVXS-101 (onasemnogene abeparvovec)
GMO Environmental Risk Assessment

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ABBREVIATIONS

AAV	Adeno-Associated Virus
ASO	Antisense Oligonucleotide
BGH	Bovine Growth Hormone
CB	Chicken-β-actin-hybrid promoter
cDNA	Complimentary Deoxyribonucleic Acid
CFU	Colony Forming Units
CMV	Cytomegalovirus
dd	Droplet Digital
DNA	Deoxyribonucleic Acid
DP	Drug product
DS	Drug substance
FBS	Foetal Bovine Serum
FDA	Food and Drug Administration
GMP	Good Manufacturing Practise
HCP	Host Cell Protein
HSPG	heparin sulfate proteoglycan
ID	Identity
ITR	Inverted Terminal Repeats
IV	Intravenous Infusion
LOQ	Limit of Quantitation
MCB	Master Cell Bank
MCS	Multiple Cloning Sites
mg	milligrams
mL	millilitre
MOI	multiplicity of infection
mRNA	Messenger Ribonucleic Acid
OD	Optical Density
pAAV	Plasmid pAAV
PCR	Polymerase Chain Reaction
pHELP	Helper Plasmid
poly A	Polyadenylation
ppm	parts per million
Q-PERT	Quantitative Product Enhanced Reverse Transcriptase
rAAV	Recombinant Adeno-Associated Virus
rcAAV	Replication Competent Adeno-Associated Virus
RNA	Ribonucleic Acid
RP-HPLC	Reverse Phase High Pressure Liquid Chromatography
sc	Self-Complimentary
SDS-PAGE	Sodium Dodecyl Sulphate PolyacrylAmide Gel Electrophoresis
SMA	Spinal Muscular Atrophy
SMN	Survival Motor Neuron
SOP	Summary of Procedure
SV	Simian virus
TFF	Tangential Flow Filtration
USP	United States Pharmacopeia
vg	vector genome
VP	Virus Particle
WCB	Working Cell Bank
wtAAV	wild-type AAV

1 INTRODUCTION

1.1 Description of the Product and Intended Release

ZOLGENSMA, onasemnogene abeparvovec (AVXS-101) is a gene therapy biological product intended for the treatment of paediatric patients diagnosed with spinal muscular atrophy (SMA), caused by mutations in the survival motor neuron 1 (*SMN1*) gene on chromosome 5q13 (5q SMA). Reported incidence of SMA in the EU is estimated to be less than 0.4/10,000 heads of population; according to latest information from 2017 there are 20,686 individuals affected by SMA in the Community. SMA is the most common inherited cause of infant mortality (Awano *et al*., 2014). There are two SMN genes, of which *SMN1* codes for a full length SMN protein and *SMN2* a truncated, unstable SMN protein (caused by alternative splicing). Mutations in the SMN genes result in decreased expression of the SMN protein (Kolb *et al*., 2015) and clinical manifestations with varying phenotypes, depending on the mutations and affected gene. The primary pathology of SMA is neurodegeneration at the level of the spinal motor neuron; however, some clinical reports indicate the involvement of the heart, liver, pancreas and intestine, and metabolic deficiencies as well (Shababi *et al*., 2014). Deficiency of the SMN protein correlates directly with death of the individual's motor neurons. Loss of motor neurons leads to secondary effects on muscle strength and function, leading to progressive loss of muscle control, strength and function, swallowing, breathing and, ultimately, death. Proximal muscles are preferentially affected in the disease, as are lower more than upper extremities (Tisdale *et al*., 2015). SMA Type 1 is characterised by hypotonia and severe weakness from early infancy. By definition, these children never attain the ability to sit without support and have a median survival (defined by the endpoint of death or a surrogate of death, the requirement of permanent ventilation) of 10.5 months (Finkel, *et al.*, 2014).

There are limited treatment options for patients with SMA. Most medicinal products have been unsuccessful in stabilising or reversing this disease. The EU Commission granted a Marketing Authorisation in May 2017 for Spinraza (nusinersen), an antisense oligonucleotide (ASO) drug designed to increase the production of the SMN protein by modulating the splicing of the *SMN2* gene, thereby compensating for the underlying genetic defect. Clinical studies with nusinersen have shown some promise in improving motor function. However, nusinersen must be administered indefinitely every 4 months via intrathecal injection and requires a lengthy induction period prior to effectiveness, and has safety considerations which require clinical monitoring.

Thus, there remains a great need for therapies that effectively target the disease mechanism.

The goal of AVXS-101 treatment is transduction of motor neurons by a viral vector containing the gene for SMN, which results in increased SMN protein expression in motor neurons, thereby preventing cell death, improving neuronal and muscular function, and increasing overall patient survival. The SMN gene present in AVXS-101 is not integrated into the patient chromosome but resides as episomal DNA in the nucleus of transduced cells and appears to be highly stable in post-mitotic cells such as motor neurons or muscle cells. Patients will receive only a single dose of AVXS-101, which is expected to lead to persistent expression of the SMN protein in the target cells.

Treatment of paediatric patients suffering from this rare inherited disease takes place in specialised hospitals/treatment centres by physicians specialised in SMA. The use of AVXS-101 gene therapy will be restricted to these expert centres. AVXS-101 will be administered via intravenous (IV) infusion. The hospital 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.

Instructions will be provided on disposal of any unused product, as well as materials used for injection, including empty product vials, sterile drapes and needles in contact with AVXS-101. All waste is advised to be sealed in leak-proof primary and secondary containers, double bagged in bags bearing the biohazard symbol and disposed in a biohazard waste container according to national/local standards for Biosafety Level 1 agents.

Patients stay in intensive care and are followed for 24 hours post administration before released home. The treated patients may transmit ZOLGENSMA after administration (see Section 3. on ZOLGENSMA shedding), leading to disposals at the treatment site and their natural environment (home etc.). However, as SMA is a rare disease, the release volumes of this GMO will remain low.

In summary, AVXS-101 is a non-replicating, non-integrating, recombinant AAV9 viral capsid shell containing the complimentary deoxyribonucleic acid (cDNA) of the human SMN gene under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB). All of the DNA from the wild-type AAV9 has been removed and replaced with the SMN genes, rendering AVXS-101 incapable of replicating itself. AVXS-101 expresses the human SMN protein in transduced cells and is used to treat patients with spinal muscular atrophy (SMA).

2 GMO CHARACTERISTICS

2.1 Identification of Characteristics Which May Cause Adverse Effects

Adeno Associated Viruses (AAV) are naturally occurring, small (20nm) non-enveloped group II viruses (*Family: Parvoviridae, Genus: Dependoparvovirus*) with a 4.7 kb single-stranded DNA genome. AAVs infect humans and wide range of animals, but are not known to cause any human pathology and have not been associated with any human or animal disease (Daya and Berns, 2008). Wild type AAV is incapable of replication without co-infection with a helper virus that can include Adenoviruses, Herpes viruses or Vaccinia.

Gene therapy vectors using AAV can infect both dividing and quiescent cells and transferred genetic material persists in an extrachromosomal state without modifying the patient's DNA (Daya and Berns, 2008). A human isolate of serotype AAV9 is used for AVXS-101 due to its´ ability to infect muscle and neuronal cells. According to published data recombinant AAV vectors enter cells via receptor-mediated endocytosis (Bartlett *et al*., 2000), where cell surface heparin sulfate proteoglycan (HSPG) serves as the primary attachment receptor for AAV (Summerford *et al*., 1999). Fibroblast growth factor receptor and $\alpha_v\beta_5$ integrin have also been implicated as co-receptors or facilitators of AAV entry into target cells (Summerford *et al*., 1998).

AVXS-101 is a non-replicating, recombinant adeno-associated virus serotype 9 (AAV9) containing the human survival motor neuron (SMN) gene under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB). 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: AVXS-101 Vector Construct

AAV2 = Adeno-associated Virus Serotype 2; BGH = Bovine Growth Hormone; cDNA = Complementary Deoxyribonucleic Acid; CB = Chicken-β-Actin Hybrid; CMV = Cytomegalovirus; ITR = Inverted Terminal Repeat; Poly A = Polyadenylation; SMN = Survival Motor Neuron; SV40 = Simian Virus 40.

The vector construct of AVXS-101 contains the SMN complementary deoxyribonucleic acid (cDNA) expression cassette flanked by adeno-associated virus serotype 2 (AAV2) inverted terminal repeat (ITR) sequences. The pSMN plasmid containing the vector construct was constructed by inserting the human SMN cDNA sequence into plasmid pAAV multiple cloning sites (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 ITRs are the AAV2 viral sequences included in this vector construct, which are required for both viral DNA replication and the packaging of the recombinant AAV (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. The capsid is comprised of 60 viral proteins (VP1, VP2, VP3) in a ratio of 1:1:10 produced by alternate splicing such that VP2 and VP3 are two truncated forms of VP1, all with common C-terminal sequences. The DNA sequence of the AVXS-101

vector construct within the pSMN plasmid is described in Table 1 and provided in Figure 2. The VP1, VP2, and VP3 amino acid sequences are provided in Figure 3 (depicted in black, purple, and blue, respectively). The AAV capsid is a 25-nm icosahedron as shown in Figure 4 (Tseng *et al.*, 2014). The predicted molecular weights of the VP1, VP2, and VP3 protein subunits are summarized in Table 2.

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

Component	Start Position	Stop Position	Size (nt)	Description	Purpose
"Left" Mutated Adeno-Associated Virus (AAV2) Inverted Terminal Repeat (ITR)	1	106	106	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 maximise vector potency, allowing lower systemic doses
Cytomegalovirus (CMV) Enhancer / Chicken-β-Actin Hybrid (CB) Promoter	153	432	280	Portion of the CMV immediate/early enhancer	Constitutive high-level SMN expression
	439	704	266	Chicken-β- actin core promoter	
Simian virus 40 (SV40) Intron	774	870	97	Intron from the SV40 (shown to enhance accumulation of steady level of mRNA for translation)	Common feature in gene vector for increased gene expression
Human Survival Motor Neuron (SMN) cDNA	1003	1887	885	Genbank Accession #NM_017411 (one nucleotide difference in relevant region)	Express full-length SMN protein
Bovine Growth Hormone (BGH) Polyadenylation (Poly A) Termination Signal	1973	2204	232	BGH poly A signal	Efficient polyadenylation of the SMN mRNA (transcription termination signal) for high-level, efficient gene expression
"Right" AAV2 ITR	2217	2359	143	Unmodified AAV2 ITR	Required in *cis* for both viral DNA replication and packaging of the rAAV vector genome

AAV2 = Adeno-associated Virus Serotype 2; BGH = Bovine Growth Hormone;
cDNA = Complementary Deoxyribonucleic Acid; CB = Chicken-β-Actin Hybrid; CMV = Cytomegalovirus;
DNA = Deoxyribonucleic Acid; ITR = Inverted Terminal Repeat; mRNA = Messenger Ribonucleic Acid;
Poly A = Polyadenylation; rAAV = Recombinant Adeno-Associated Virus; SMN = Survival Motor Neuron;
SV40 = Simian Virus 40.

Figure 2: **AVXS-101 Vector Construct DNA Sequence (colors of the sequence correspond to Table 1)**

```
ctgcgcgctc gctcgctcac tgaggccgcc cgggcaaagc ccgggcgtcg 50
ggcgaccttt ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg 100
gagtggaatt cacgcgtgga tctgaattca attcacgcgt ggtacctctg 150
gtcgttacat aacttacggt aaatggcccg cctggctgac cgcccaacga 200
cccccgccca ttgacgtcaa taatgacgta tgttcccata gtaacgccaa 250
tagggacttt ccattgacgt caatgggtgg agtatttacg gtaaactgcc 300
cacttggcag tacatcaagt gtatcatatg ccaagtacgc cccctattga 350
cgtcaatgac ggtaaatggc ccgcctggca ttatgcccag tacatgacct 400
tatgggactt tcctacttgg cagtacatct actcgaggcc acgttctgct 450
tcactctccc catctccccc ccctccccac ccccaatttt gtatttattt 500
atttttaat tattttgtgc agcgatgggg gcgggggggg gggggggcg 550
cgcgccaggc ggggcggggc ggggcgaggg gcggggcggg gcgaggcgga 600
gaggtgcggc ggcagccaat cagagcggcg cgctccgaaa gtttcctttt 650
atggcgaggc ggcggcggcg gcggccctat aaaaagcgaa gcgcgcggcg 700
ggcggagcg ggatcagcca ccgcggtggc ggcctagagt cgacgaggaa 750
ctgaaaaacc agaaagttaa ctggtaagtt tagtcttttt gtcttttatt 800
tcaggtcccg gatccggtgg tggtgcaaat caaagaactg ctcctcagtg 850
gatgttgcct ttacttctag gcctgtacgg aagtgttact tctgctctaa 900
aagctgcgga attgtacccg cggccgatcc accggtccgg aattcccggg 950
atatcgtcga cccacgcgtc cgggccccac gctgcgcacc cgcgggtttg 1000
ctatggcgat gagcagcggc ggcagtggtg gcggcgtccc ggagcaggag 1050
gattccgtgc tgttccggcg cggcacaggc cagagcgatg attctgacat 1100
ttgggatgat acagcactga taaaagcata tgataaagct gtggcttcat 1150
ttaagcatgc tctaaagaat ggtgacattt gtgaaacttc gggtaaacca 1200
aaaaccacac ctaaaagaaa acctgctaag aagaataaaa gccaaaagaa 1250
gaatactgca gcttccttac aacagtggaa agttggggac aaatgttctg 1300
ccatttggtc agaagacggt tgcatttacc cagctaccat tgcttcaatt 1350
gattttaaga gagaaacctg tgttgtggtt tacactggat atggaaatag 1400
agaggagcaa aatctgtccg atctactttc cccaatctgt gaagtagcta 1450
ataatataga acagaatgct caagagaatg aaaatgaaag ccaagtttca 1500
acagatgaaa gtgagaactc caggtctcct ggaaataaat cagataacat 1550
caagcccaaa tctgctccat ggaactcttt tctccctcca ccacccccca 1600
tgccagggcc aagactggga ccaggaaagc caggtctaaa attcaatggc 1650
ccaccaccgc caccgccacc accaccaccc cacttactat catgctggct 1700
gcctccattt ccttctggac caccaataat tcccccacca cctcccatat 1750
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tggtacatga gtggctatca tactggctat tatatgggtt ttagacaaaa 1850
tcaaaaagaa ggaaggtgct cacattcctt aaattaagga gaaatgctgg 1900
catagagcag cactaaatga caccactaaa gaaacgatca gacagatcta 1950
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gccttctagt tgccagccat ctgttgtttg cccctccccc gtgccttcct 2050
tgaccctgga aggtgccact cccactgtcc tttcctaata aaatgaggaa 2100
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ggggcaggac agcaaggggg aggattggga agacaatagc aggcatgctg 2200
gggagagatc gatctgagga acccctagtg atggagttgg ccactccctc 2250
tctgcgcgct cgctcgctca ctgaggccgg gcgaccaaag gtcgcccgac 2300
gcccgggctt tgcccgggcg gcctcagtga gcgagcgagc gcgcagagag 2350
ggagtggcc                                            2359
```

Figure 3: **AAV Capsid Proteins: VP1, VP2, VP3 Sequences**

VP1, VP2 and VP3 are derived from the same transcript but have alternative start sites but share a carboxy terminus. VP1 specific amino acids are shown in black. Amino acids common to VP1 and VP2 are shown in purple. Amino acids common to all three capsid proteins are shown in blue.

```
  1  MAADGYLPDW LEDNLSEGIR EWWALKPGAP QPKANQQHQD NARGLVLPGY KYLGPGNGLD

 61  KGEPVNAADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF QERLKEDTSF GGNLGRAVFQ
                                     VP1

121  AKKRLLEPLG LVEEAAKTAP GKKRPVEQSP QEPDSSAGIG KSGAQPAKKR LNFGQTGDTE
                                           VP2

181  SVPDPQPIGE PPAAPSGVGS LTMASGGGAP VADNNEGADG VGSSSGNWHC DSQWLGDRVI

241  TTSTRTWALP TYNNHLYKQI SNSTSGGSSN DNAYFGYSTP WGYFDFNRFH CHFSPRDWQR

301  LINNNWGFRP KRLNFKLFNI QVKEVTDNNG VKTIANNLTS TVQVFTDSDY QLPYVLGSAH

361  EGCLPPFPAD VFMIPQYGYL TLNDGSQAVG RSSFYCLEYF PSQMLRTGNN FQFSYEFENV

421  PFHSSYAHSQ SLDRLMNPLI DQYLYYLSKT INGSGQNQQT LKFSVAGPSN MAVQGRNYIP
                                     VP3

481  GPSYRQQRVS TTVTQNNNSE FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS

541  LIFGKQGTGR DNVDADKVMI TNEEEIKTTN PVATESYGQV ATNHQSAQAQ AQTGWVQNQG

601  ILPGMVWQDR DVYLQGPIWA KIPHTDGNFH PSPLMGGFGM KHPPPQILIK NTPVPADPPT

661  AFNKDKLNSF ITQYSTGQVS VEIEWELQKE NSKRWNPEIQ YTSNYYKSNN VEFAVNTEGV

721  YSEPRPIGTR YLTRNL
```

Figure 4: **The AAV Capsid**

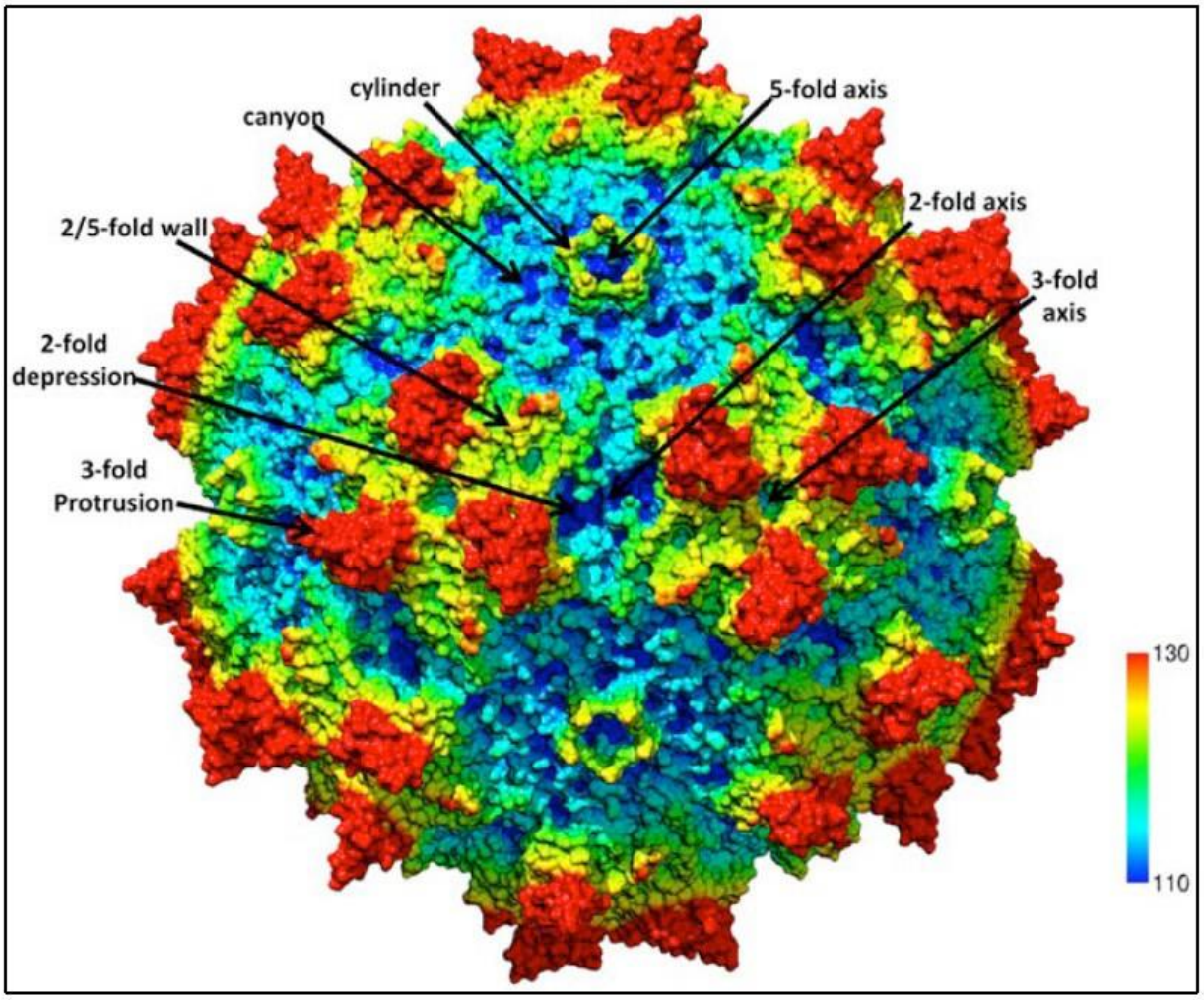

Table 2: **Predicted VP1, VP2 and VP3 Molecular Weights**[1]

Component	Molecular Weight
VP1	81.4 kDa
VP2	66.3 kDa
VP3	59.8 kDa

VP = Viral Protein

1 Predicted molecular weights calculated with ExPASy calculator using VP1, VP2, and VP3 sequences in Figure 3.

For manufacturing purposes, the non-replicative recombinant AAV9 viral vector is packaged using a helper-virus free system comprising of the following GMP qualified plasmid DNA:

1) pSMN vector plasmid
2) AAV9 plasmid termed pAAV2-9 containing the AAV *rep2* and *cap9* wild-type genes
3) Helper adenovirus plasmid pHELP

Plasmids are transfected into HEK 293 producer cells from GMP qualified Master Cell Banks that have been extensively tested for adventitious agents and found suitable for commercial production.

The characteristics of AVXS-101 that could cause adverse effects include:

- replication competent viruses as impurities (helper viruses / adventitious agents from raw and starting materials)
- viral sequences associated with infectious diseases
- capability of the recombinant virus to spread
- capability to transfer genetic material to other species
- adverse degradation products
- expression of the SMN protein in transduced cells.

In conclusion, AVXS-101 is a non-replicating AAV9 vector containing an *SMN* transgene for expression of the SMN protein. As the starting materials and some raw materials used in production of AVXS-101 are of biological origin, there are possibilities for contamination with replication competent viruses, that could act also as helper viruses for recombination *in vivo*. The vector construct contains elements from viruses that are associated with infectious diseases and it includes an antibiotic resistance gene, that could be transmitted to the environment. Degradation of the vector might produce harmful compounds and high-level expression of the transgene might be of concern.

3 ENVIRONMENTAL ADVERSE EFFECTS

Potential environmental adverse effects associated with *viral vectors* usually include spreading, infection, pathogenicity, integration, transmission and immune responses. Viral vectors, depending on the modification, may also transfer genetic material to other species, undergo genetic or phenotypic changes, remain latent or produce harmful degradation products.

AVXS-101 is a non-replicating, recombinant adeno-associated virus serotype 9 (AAV9) containing the human survival motor neuron (SMN) gene under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB). AAV serotype 9 is a naturally occurring human AAV (Clade F) isolated from healthy human donor tissue (Gao *et al.*, 2004). AAVs, including AAV2 and AAV9 are non-pathogenic and thus do not cause any know disease in humans. These dependo-virus require the presence of a helper virus for replication (Tenenbaum 2003).

Wildtype AAV has not been classified under Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. Consequently, AAV fulfils the definitions of group 1 Biological agent according to the criteria specified in Directive 2000/54/EC (a biological agent that is unlikely to cause human disease). The wild type virus is incapable of independent replication and requires co-infection of a helper virus to enable a lytic replication cycle (Gonclaves, 2005).

Recombinant AAV viral vector (rAAV) used for production of ZOLGENSMA has been engineered to contain only the cis-acting Inverted Terminal Repeat (ITR) regions from the parental AAV2 virus and has no other viral DNA sequences remaining. The vector is incapable of replication and exists as episomal DNA in transduced cells with no targeted integration into the host genome. None of the sequences of the inserted transgene are known to have pathogenic or harmful characteristics. The CMV enhancer and the SV40 intron are separate recombinant units widely used in molecular engineering and not capable of mediating the harmful characteristics of the corresponding parental viruses. As the vector construct is replication defective, even in the presence of a helper virus, no new progeny viruses are being produced, Thus, the pathogenicity of AVXS-101 is expected to be even less than that of AAV2 or AAV9 viruses, which are already considered non-pathogenic (Directive 2000/54/EC).

Dissemination of AVXS-101 would most likely only occur between unintended human recipients since it is derived from AAV2/9. The most likely potential scenarios in which AVXS-101 may disperse from patients into the environment (e.g. healthcare workers and close contacts of the patients), are via a spill, a needle stick injury during IMP administration, via blood following needle stick injury or via shedding directly from the patient. AveXis Inc. has conducted a shedding study in humans, where AVXS-101 was analysed from urine, stool, and saliva samples of 5 treated patients. All five patients analysed were dosed IV with the proposed therapeutic dose equivalent to 1.1×10^{14} virus genomes (vg)/kg. For the analysis of the product, a validated scAAV9-SMN Genomic Titre Assay using Droplet Digital Polymerase Chain Reaction (ddPCR) is utilised. According to the results, AVXS-101 is detectable in the shed samples from day 1 post injection. AVXS-101 concentrations in urine and saliva were 0.1% to 0.01% of initial concentration in the body at day 1 post-infusion, after which concentrations fell below the limit of quantitation. In stool, levels 10% to 30% of the initial concentration in the body were detectable at day 1 post-infusion. One patient showed a peak concentration in stool at day 14 post-infusion of 280% of initial concentration in body. In contrast, 3 patients for whom data were available showed a concentration of

< 1% of initial concentration in the body at day 14 post-infusion, with concentrations declining approximately 4 logs (10,000-fold) over 30 days post-infusion. Overall, ZOLGENSMA was primarily cleared from the body in stool and the levels were below the limit of quantitation (1.1×10^6 GC/g) by day 60 post-infusion.

The findings of the shedding study are in line with published data demonstrating that shedding of rAAV vector DNA can be detected for a number of weeks from patient excrements (Favre *et al.*, 2001; Manno *et al.*, 2006; Provost *et al.*, 2005). Shedding is reported to be dependent on the dose and route of administration; the IV route can be considered a worstcase scenario for AAV shedding. However, even in the case of shedding, the AAV vectors do not propagate outside of cells (Schenk-Braat et al., 2007; Afione et al., 1996).

The viral vector may be transmitted also to non-target subjects via spills/splashes and/or needle sticks during the treatment procedure. The risk and consequences of vector transferred from an accidental needle stick will be considered in two scenarios: 1) where a healthcare worker accidentally is stuck by the needle during AVXS-101 administration and 2) where the healthcare worker is stuck by a needle exposed to patient blood. In the first scenario, the worker would have the potential to be exposed to a full dose of AVXS-101. If the worker is seropositive for AAV9 antibodies, the most likely scenario is a neutralization of the incoming virus resulting in negligible infection. In the scenario where the worker is seronegative for AAV9 antibodies, it is analogous to an SMA patient receiving a subtherapeutic dose due to the large difference in body weight between an SMA infant and an adult healthcare worker. Since AAV9 is non-pathogenic in humans and SMN overexpression is also not associated with pathology, the risks and consequences to a healthcare worker would be less than an SMA patient receiving treatment due to the decrease in relative dose. The consequences of AVXS-101 exposure posed to a healthcare worker by a needle stick event with patient blood are negligible. rAAV vectors have low infectivity and require high virus titers for efficient transduction of cells. Thus, virus particles from the patients hardly have the capacity to cause significant infections.

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 virus. If this happens, the AAV9 vector could form a virus that causes infection if the healthy unintended human recipient 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 would be cleared by the healthy unintended human recipient immune system. 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. 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 wt AAV and adenovirus were administered in a particular setting (Afione *et al*., 1996). As mentioned above, the manufacturing process utilises a tri-plasmid transfection system, where no helper viruses are used and all starting and raw materials are tested for adventitious agents. It is recognised that the risk of recombination exists with all transfection systems. However, with a three plasmid system, the probability of the risk approaches zero. The three plasmid production system for AAVs was first introduced in 1998 (Xiao *et al*., 1998) and since then there have not been any reported cases of *in vivo* recombination when using this approach. Taken together, AAV9 interaction with other viruses to cause infection appears to be a minimal risk for AVXS-101.

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 AA V. The transgene (human survivor motor neuron) is not expected to confer any advantage to the genetically modified viral vectors in terms of survival and selective pressure. 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 genetically modified viral vectors (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.

As described above, genetic engineering of AVXS-101 makes it incompetent to replicate. The alterations made to the vector backbone and the added transgene are not known to change the host range or tropism of rAAV9, for which no environmental risks have been reported. The transgene is the recombinant human counterpart of *SMN* gene and due to the engineered promoter it is not expressed from the vector outside eukaryotic cell nucleus. Thus, even if shed to the environment, the vector and the transgene are not functional and able to cause any toxicities to non-target humans, animals or plants. Furthermore, based on non-clinical and clinical studies, AVXS-101 is not known to be pathogenic or associated with animal, plant or microbial toxicities

There is a theoretical risk that AAV infection could lead to 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). 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. Although AVXS-101 is not anticipated to integrate into the host cell genome, the long-term consequences of administering AAV viral vectors to humans are not yet fully understood. However, recombinant AAVs do not have the ability to stably integrate into the host cell genome at a specific site (designated AAVS1) in the human chromosome 19, as wild-type AAVs, which also are non-pathogenic despite of the integration (Kotin, *et al*., 1990; Surosky, *et al*., 1997). As ZOLGENSMA does not integrate into the patient genome, germ-line cells are not impacted and risk for germline transmission is negligible.

The wild type AAV does not contain any gene that confers resistance to known antibiotics. However, the plasmid selection system of the production process utilises Kanamycin resistance gene and it is recognised that the sequence can be co-packed into the virus particles during production. To mitigate this risk, the presence of this possible impurity is analysed as part of the AVXS-101 Drug Substance (DS) release testing. This residual plasmid DNA test is a qPCR based assay specific to the Kanamycin resistance gene region of the plasmid, measuring both free and encapsulated plasmid DNA.

Recombinant, replication deficient AAVs are not known to be able to transmit genetic material and no natural mobile genetic elements such as transposons or plasmids have been reported for AAVs. Due to the genetic modification, which has removed all AAV viral elements except the AAV2 ITRs, the possibility of transfer of genetic material and

transmission are very low. The only viral element associated with infectious diseases is the SV40 intron, which is widely used in recombinant viral vectors and is not capable of transmitting any viral functions of the SV40 virus.

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. The genetic modification has not changed the infectivity, tropism or host range of wild type AAV9. Due to the lack of rep and cap genes, AVXS-101 is replication incompetent even in the presence of a helper virus. The vector product has been found stable during storage (up to 24 months when stored frozen) and after administration to the patients, thus the risk of harmful degradation products is negligible. There are no known toxicities related to the expressed SMN protein, if released to the environment from transduced cells.

There are conflicting reports that integration of the wild-type AA V2 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 in Europe and the US with no known disease or pathogenic effects (Boutin 2010) 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. For a review of the topic, see 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.

A recent review of viral shedding from clinical gene therapy trials reported PCR-based assays were used in 7 other studies of AAV-based therapies that were surveyed, including samples from 84 separate patients (Schenk-Braat et al.). None of these studies detected intact virus (Ibid.), and thus to our knowledge there remains no evidence in favour of intact viral shedding in AAV-based clinical gene therapy trials. The same review noted that even in clinical gene therapy trials based on other, non-AAV viral delivery systems, shedding of infectious viral particles is uncommon, and was reported in only 7 out of 92 trials, all of which utilized a completely different adenoviral vector (Ibid.) disease (Tenenbaum et al., Salganik et al.)

Propagation of rAAV vectors has not been demonstrated in humans. In experimental settings, transient propagation of rAAV vectors was demonstrated in only a single study (Afione et al.) and required direct inoculation of the respiratory epithelium with a high concentration of virus. Recovery of the engineered vector was a transient phenomenon, lasting no more than 14 days (Afione et al.). It is exceedingly unlikely that a similar level and route of contamination would occur outside a laboratory setting. In the case of AVXS-101, vector shedding is limited in time and occurs mainly through the feces. This makes propagation unlikely as it would require fecal inoculation of a host with a significant oral volume of the patient's feces. Simple contact precautions as recommended in the SPC and PIL are expected to adequately prevent this unlikely scenario.

Even-though it is not expected that the transmission of the vector would lead to adverse effects in healthy humans since neither wild type AA V nor A VXS-101 are known to be pathogenic, instructions for such situations should be followed. (See Section 7).

In summary, while AVXS-101 is capable of infecting various cells, the virus particles are not able to multiply outside of cells. Shedding of the vector is taking place mainly through

faeces of the treated patients; however, the shedding continues only for a short time period (max 60 days) and can be controlled with proper handling of the faeces (see Section 7 for mitigation activities). AVXS-101 is a non-pathogenic and non-integrating vector with no known capabilities to transfer genetic material to other species. The production system involves a kanamycin resistance gene, which could be co-packed as an impurity into the product and thus be released to the patients and the environment. However, this impurity is controlled as part of the DS testing. Thus, the overall environmental risk is considered negligible.

3.1 Potential Consequences of Adverse Effects

As AVXS-101 is replication incompetent, non-pathogenic, non-integrating vector and not known to have any adverse environmental effects, potential consequences of the release are considered minimal. The risks for shedding, recombination, degradation and transfer of antibiotic resistance genes are controlled during quality testing (see Section 5 below).

3.2 Magnitude of Each Identified Consequence

The magnitude and severity of environmental effects for AVXS-101 are considered negligible and self-limiting, due to the following reasons:

- ZOLGENSMA is a non-replicating, non-integrating, recombinant AAV9 viral capsid shell containing the complimentary deoxyribonucleic acid (cDNA) of the human SMN gene under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB). All of the DNA from the wild-type AAV9 has been removed and replaced with the SMN genes, rendering AVXS-101 incapable of replicating itself. The risk of spreading of the genetically modified vector to the environment is very small, as it is highly unlikely that a triple infection (AAV vector, wildtype AAV en helpervirus) would occur.
- There are no known susceptible species that would be at risk due to the shedding of the product. AAV9 is not known to cause any toxicities to humans, animals or plants and the recombinant human SMN protein is expressed from the transgene only in the nucleus of eukaryotic cells. In addition, the transgene is not known to change the host range or tropism of AAV9, neither does it give any growth/propagation advantage for the vector.
- rAAV vectors have low infectivity and require high virus titres for efficient transduction of cells. Thus, virus particles excreted from the patients hardly have the capacity to cause significant infections in humans, animals or plants.
- Given that the rAAV9 vector is completely non-replicative and is not a known pathogen of any plant or animal species and given that the wild type virus is naturally occurring in the environment, the risk to the environment from exposure to potentially contaminated material is considered to be minimal.
- Shedding of the vector from the patients is limited in time and mainly occurring through the faeces. The clinical use is for a rare disease in a very limited number of patients and occurs primarily in centres of excellence in the care of patients with SMA.
- ZOLGENSMA will be prepared in a Class II Biological Safety Cabinet by a trained pharmacist and the drug will be transported to the patient's room in a leak-proof container limiting the potential for spills to the environment.
- Healthcare workers are instructed to wear appropriate personal protective equipment in accordance with BSL 1 and follow strict good hand-hygiene practices. The

treatment centers also instructed to follow site, local and country requirements for disposal of viral waste material (see Section 7), thus further reducing the possible transmission to others and/or the environment.

- AVXS-101 is intended for treatment of spinal muscular atrophy, a rare inherited disease. According to published data, the prevalence of the disease is less than 0.4 / 10,000 heads of population and thus the clinical use of the product will remain highly limited.
- AAV viral capsids are known to be stable; however, the virus is sensitive to readily available disinfectants for non-enveloped viruses such as 10% solution of chlorine bleach or oxidizing agents and can be eliminated through defined sanitation procedures.
- The risks relating to presence of replication competent viruses and co-purified genetic elements are mitigated through DP testing.
- Although studies evaluating AA V viral vector bio-distribution and shedding in non-human primates and in human subjects enrolled in clinical trials utilizing rAAV vectors indicate the levels of shed vector present in bodily fluids rapidly declined to undetectable levels within days or weeks post administration (Manno et al. 2006; Favre et al. 2001; Salmon et al. 2014), staff should adhere to local Health and Safety guidelines applicable to biological sample collection, processing, and shipment.
- Given the low number of patients expected to be exposed since SMA is a rare disease and the level of expertise and training of the medical personnel allowed to manipulate the IMP, and to obtain patient samples, it is very unlikely that the GMO will spread from the test subject into the environment as the levels of the GMO in the blood of the treated patient are barely detectable and the route of administration poses a negligible risk of shedding from patients. The infectivity risk is low as AVXS-101 is a non-replicating recombinant adeno-associated virus.
- Given that the rAAV2/9 vector used is completely non-replicative and is not a known pathogen of any plant or animal species and given that the wild type virus is naturally occurring in the environment, the risk to the environment from exposure to potentially contaminated material is considered to be minimal.
- In order to minimize risk to non-intended recipients patients are hospitalized for up to 48 hours following gene replacement therapy administration. During the inpatient stay, personnel are required to follow appropriate safety precautions as per institutional standards for infection control; standards should require personal protective equipment (PPE} such as gowns and gloves. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved instructions are 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.
- ZOLGENSMA is administered in a controlled environment, where access to non-humans is limited. In addition, vector shedding from subjects is negligible and no selective advantages or disadvantages are conferred over untreated individuals.
- No interactions between the ZOLGENSMA and non-target organisms are expected given the quantities involved, the nature of the release and the non-replicative nature of ZOLGENSMA.

- Immediate and/or delayed effects on biogeochemical processes are not expected. As 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 I, it is not deemed to have an impact on the biogeochemical processes.

3.3 Likelihood of Occurrence of each Identified Potential Adverse Effect

ZOLGENSMA is a non-replicating vector and the administration of the product to patients is associated with limited exposure of the environment to the vector. Thus, exposure of plants or animals is not expected, and the environmental impacts are considered negligible.

Shedding of the vector is demonstrated in the completed and ongoing clinical studies conducted by AveXis Inc. However, as there are no adverse consequences of this spreading, the effect is considered negligible. The likelihood of recombination is very low, due to the characteristics of the vector and proper control for replication competent viruses. In addition, the probability that genetic material would be transmitted from the product is very low due to the fact that there are no mobile elements involved and co-purification of possible genetic impurities (mainly kanamycin resistance gene) is controlled for every batch.

4 ESTIMATION OF THE RISK POSED BY EACH IDENTIFIED CHARACTERISTIC OF THE GENETICALLY-MODIFIED VIRAL VECTOR

The risks posed by the identified characteristics of the AVXS-101 vector are listed in Table 3. together with evaluation of the magnitude and likelihood of each risk. The evaluation provides an overall estimate for each identified risk.

Table 3: Estimation of the risk to human health and the environment posed by each identified characteristic of the GMO

Adverse effects	Magnitude of an adverse effect	Likelihood of occurrence of an AE	Outcome of the risk estimate
Genetic/phenotypic change	Low	Low	Low
Reproduction	Negligible	Negligible	Negligible
Spreading	Low	Unlikely	Negligible
Infectious disease	Negligible	Negligible	Negligible
Latency	Negligible	Negligible	Negligible
Recombination	Negligible	Negligible	Negligible
Insertional mutagenesis	Negligible	Negligible	Negligible
Germline transmission	Negligible	Negligible	Negligible
Transfer of genetic material to other species (packing of impurities)	Low	Low	Low
Degradation	Low	Low	Low

5 MANAGEMENT STRATEGIES FOR RISKS FROM USE OF THE GENETICALLY-MODIFIED VIRAL VECTORS

5.1 Starting Materials of Biological Origin

For manufacturing purposes, the non-replicative recombinant AAV9 viral vector is packaged using a helper-virus free system comprising of the following GMP qualified plasmid DNA:

- Vector Plasmid (pSMN, formerly pscAAV.CB.SMN)
- AAV Plasmid (pAAV2/9) containing the AAV rep2 and cap9 wild-type genes
- Adenovirus Helper Plasmid (pHELP)

Plasmids are transfected into HEK293 producer cells from GMP qualified Working Cell Banks (WCBs) that have been extensively tested for adventitious agents and found suitable for commercial production.

With this approach, no helper viruses are needed for production of the recombinant virus and contamination of AVXS-101 Drug Product (DP) with helper viruses is avoided.

It is recognized that the risk of recombination exists with all transfection systems, however, with a three plasmid system, probability of the risk approaches zero. The three plasmid production system for AAVs was first introduced in 1998 (Xiao et al., 1998) and since then there have not been any reported cases of *in vivo* recombination when using this approach.

The plasmid selection system utilizes a kanamycin resistance gene and it is recognized that the sequence can be co-packed into the virus particles during production. The presence of this possible impurity is analyzed as part of the commercial release specification for AVXS-101 Drug Substance (DS).

This residual plasmid DNA test is a qPCR based assay specific to the kanamycin resistance gene region of the plasmid, measuring both free and encapsulated plasmid DNA. The results of the batch analyses are presented in Table 4. The proposed commercial specification acceptance criterion is ≤ 1.4E6 pg per 1.0E13 vg per 1 x 10^{13} vg.

Table 4: Residual Plasmid DNA Batch Analysis Results for AVXS-101 Lots Released for use in the Clinical Program

Attribute	Test Method	Proposed Commercial Acceptance Criterion	Lot Number	Manufacturing Date	Release Result
Residual Plasmid DNA by qPCR	SOP-186	≤ 1.4x 10^{6} pg per 1.0x10^{13} vg	NCH Phase 1 Lot AAV9SMN0613	18-Dec-2013	3.0 × 10^{12} copies/mL[a]
			AVXS-101 DP Lot 816836[C]	01-Jun-2017	8.1x10^{5} pg/mL per 1.0x10^{13} vg/mL[b]
			AVXS-101 DP Lot 600156	07-Nov-2017	9.5x10^{5} pg per 1.0x10^{13} vg
			AVXS-101 DP Lot 600307	07-Nov-2017	1.2x10^{6} pg per 1.0x10^{13} vg
			AVXS-101 DS Lot 600409	12-Dec-2017	5.7 x 10^{5} pg per 1.0x10^{13} vg
			AVXS-101 DS Lot 600410	19-Dec-2017	4.3 x 10^{5} pg per 1.0x10^{13} vg
			AVXS-101 DS Lot 600454	08-Jan-2018	9.1 x 10^{5} pg per 1.0x10^{13} vg

a. NCH residual plasmid DNA assay targets different antibiotic resistance sequences in the plasmids used in manufacturing. Original Phase 1 Lot release value reported as 3.0 × 10^{12} copies/mL.

b. Adjusted result. Actual values have been multiplied by 1/5.30 to provide values per 1.0x10^{13} vg/mL.

For AVXS-101 DS, all plasmid preparations and raw materials are tested for possible adventitious agents and the plasmid MCBs have been fully characterized and tested for possible microbiological impurities. For the commercial production of AVXS-101 DS, the plasmids are manufactured at both Cobra Biologics Ltd (Cobra) and Ajinomoto Althea, Inc. (Althea). A third manufacturer, VGXI, Inc., will be qualified as an additional commercial manufacturer of plasmids according to AveXis' prospective Plasmid Qualification Plan (PLAN-296).

The analytical procedures utilized for the plasmid testing for possible adventitious agents together with specifications and acceptance criteria are presented in Table 5 and Table 6 for Cobra and Althea, respectively.

Table 5: Commercial – Cobra Plasmid DNA Specifications

Test	Method	Laboratory	pSMN Specification	pAAV2/9 Specification	pHELP Specification
Residual Rnase A	SDS-PAGE	Cobra	≤ 1% w/w	≤ 1% w/w	≤ 1% w/w
Endotoxin	Kinetic Quantitative Limulus Amebocyte Lysate	Cobra	< 10 EU/mg	< 10 EU/mg	< 10 EU/mg
Bioburden	Membrane Filtration	Cobra	< 1 CFU / 10 mL	< 1 CFU / 10 mL	< 1 CFU / 10 mL
Residual Kanamycin	Standard Agar Diffusion Test	Cobra	< 5 ppm	< 5ppm	< 5 ppm
Residual gDNA	qPCR	Cobra	≤ 5% (w/w gDNA)	≤ 5% (w/w gDNA)	≤ 5% (w/w gDNA)
Residual E. Coli HCP	ELISA	Cobra	≤ 1% (w/w HCP)	≤ 1% (w/w HCP)	≤ 1% (w/w HCP)
Residual RNA	RP-HPLC	Cobra	≤ 1% (w/w RNA)	≤ 1% (w/w RNA)	≤ 1% (w/w RNA)
Minimum 2-fold Sequencing Bidirectional Read	Sanger Sequencing	Eurofins	100% Identical to Reference Sequence	100% Identical to Reference Sequence	100% Identical to Reference Sequence

Table 6: Commercial – Althea Plasmid DNA Specifications

Test	Method	Laboratory	pSMN Acceptance Criteria	AAV2/9 Acceptance Criteria	pHELP Acceptance Criteria
Residual RNA	HPLC	Althea	≤ 1.0% peak area (LOQ: 1.0%)	≤ 1.0% peak area (LOQ: 1.0%)	≤ 1.0% peak area (LOQ: 1.0%)
Residual Protein	BCA	Althea	≤ 1.0% w/w (LOQ: 1 µg/mL)	≤ 1.0% w/w (LOQ: 1 µg/mL)	≤ 1.0% w/w (LOQ: 1 µg/mL)
Endotoxin	USP <85>	Althea	< 10 EU/mg	< 10 EU/mg	< 10 EU/mg
Bioburden	USP <61>, Membrane Filtration	Althea	≤ 1 CFU/10mL	≤ 1 CFU/10mL	≤ 1 CFU/10mL
Residual Kanamycin	Standard Agar Diffusion Test	Cobra	< 5 ppm	< 5ppm	< 5 ppm
Residual E. Coli DNA	qPCR	Avance Biosciences	≤ 1.0% w/w	≤ 1.0% w/w	≤ 1.0% w/w

Cell Banks

The HEK293 MCB and Working Cell Banks (WCBs) are fully tested for microbiological impurities. Thus, the environmental risks posed by the starting materials are considered negligible.

5.2 Raw materials

Raw materials of biological origin that might contribute to the risk of environmental effects include fetal bovine serum (FBS) and OptiMEM, which are fully tested for microbiological impurities.

With appropriate sourcing, controlling, and testing of raw materials, the risk of adventitious agent contamination is minimized. Extensive testing of the MCB, WCB, and unprocessed bulk harvest provides confirmation that the cell substrate and starting materials for the AVXS-101 DS manufacturing process are free of detectable adventitious agents. Viral clearance studies using model viruses demonstrate that the AVXS-101 DS purification process is able to provide sufficient capacity to remove/inactivate viruses with broad ranges of biochemical and biophysical properties. Testing of the cells beyond the limit of *in-vitro* cell age allows assessment as to whether expression of endogenous retroviruses is induced due to the production process and provides assurance that the production process is not prone to contamination by adventitious viruses. Together, the combination of these measures maximizes the safety assurance of AVXS-101 DS and reduces the risk to the environment.

5.3 Batch Analyses

The viral vector harvested from the HEK293 cell lysate is purified using Cation Exchange Chromatography, Tangential Flow Filtration 2 (TFF2), Ultracentrifugation with Cesium Chloride Density Gradient and Tangential Flow Filtration 3 (TFF3). Testing for viral contaminants or possible viral vector related impurities/degradation products that could contribute to the environmental risks is performed as follows:

- In-process testing of production control cells for viral contaminants by *in vitro* assay
  - All lots met the acceptance criterion of “none detected”
- In-process testing of the pre-lysate harvest for sterility per Ph.Eur.2.6.1.
  - All lots met the acceptance criterion of “no growth”
- AVXS-101 DS release testing for Residual Plasmid DNA by qPCR
  - All lots met the acceptance criterion.
- AVXS-101 DP release testing for Replication Competent AAV by Cell-based Assay and TaqMan® qPCR
  - All lots met the acceptance criterion.
- AVXS-101 DP release testing for % Capsid Distribution by SV-AUC
  - All lots met the acceptance criterion.

Empty capsids are not considered to pose any risk to the environment, nor does the minimal plasmid impurities detected as co-packed into the viral vector particles. No degradation products or significant impurities have been found that would pose a risk to the environment.

Only lots successfully meeting full specifications for AVXS-101 DS and AVXS-10 DP are released.

5.4 Analytical Methods

Methods used to detect any viral contaminants or possible impurities/degradation products that could contribute to the environmental risks are as follows:

Viral Contaminants by *in vitro*

The method for Viral Contaminants by *in vitro* for AVXS-101 Production Control Cells and Pre-Lysed Harvest is performed according to SOP-375. Product samples are inoculated into six-well culture plates (one per article) containing MRC-5 cells, Vero cells, and one or more additional indicator cells. The culture plates are maintained for 14 days and examined microscopically for the appearance of viral cytopathic effects weekly. The cultures are tested on day 14 for hemadsorption and hemagglutination activity using chicken, guinea pig and rhesus monkey erythrocytes. The product samples are passaged on day 14 by collecting conditioned medium from the original 6-well plates and inoculated into fresh plates of each indicator cell line. The cultures are tested on day 28 for hemadsorption and hemagglutination activity using chicken, guinea pig and rhesus monkey erythrocytes. The method has been validated and is being performed under GMP conditions.

Sterility per Ph.Eur.2.6.1.

The method for Sterility testing for AVXS-101 Pre-Lysed Harvest is performed using the membrane filtration method in accordance with Ph.Eur.2.6.1. The method has been validated and is being performed under GMP conditions.

Residual Plasmid DNA by qPCR

The presence of Residual Plasmid DNA in AVXS-101 DS is determined using a quantitative Polymerase Chain Reaction (qPCR) assay specific to the Kanamycin resistance gene region of the plasmid, measuring both free and encapsulated plasmid DNA. The method has been validated and is being performed under GMP conditions.

Replication Competent AAV by Cell-based Assay and TaqMan® qPCR

Replication competent AAV (rcAAV) testing of AVXS-101 DS is measured using a two-part passage method consisting of HEK-293 cell-based virus amplification and passage followed by detection of rcAAV deoxyribonucleic acid (DNA) by TaqMan® quantitative polymerase chain reaction (qPCR) to amplify and detect AAV2 Rep sequence DNA. The method has been validated and is being performed under GMP conditions.

% Capsid Distribution by SV-AUC

The % Capsid Distribution of AVXS-101 DP is determined using a sedimentation velocity analytical ultracentrifugation (SV-AUC) method. The SV-AUC technique combined with Sedfit analysis and Chromatography Data Software (CDS) integration are used for the purpose of measuring the sedimentation behavior of AVXS-101 DP samples. The data are used to derive (1) the distribution profiles of all species present in the sample, (2) the sedimentation coefficient values (S-values) and area percentages of empty and full virions, and (3) the relative distribution or ratio of empty-to-full AAV virions. The method has been validated and is being performed under GMP conditions.

5.5 Description of the Dosage Form

AVXS-101 DP is a single-dose, preservative-free, sterile, clear to slightly opaque, and colorless to faint white, intravenous infusion of non-replicating, self-complementary AAV9 vector at a target concentration of 2.0 x 10^{13} vg/mL. Each 1 mL of AVXS-101 DP

solution in Water for Injection (WFI) contains 20 mM Tromethamine (Tris), 1 mM Magnesium Chloride, 200 mM Sodium Chloride, and 0.005% w/v Poloxamer 188. The pH range of the solution is 7.7 to 8.3. AVXS-101 DP is filled into 10 mL crystal zenith vials with a nominal fill volume of 5.5 mL or 8.3 mL and stored at ≤ -60°C. The composition of one vial of AVXS-101 DP is presented in Table 7. Each vial also includes a target overfill of 0.4 mL. The nominal batch size is up to 2 L.

Table 7: Composition of AVXS-101 Drug Product

Component	Quality Standard
AVXS-101 Drug Substance	In-House Standard
Tromethamine	BP, JP, Ph. Eur., USP
Magnesium Chloride	BP, JP, Ph. Eur., USP
Sodium Chloride	BP, JP, Ph. Eur., USP
Poloxamer 188	NF, Ph. Eur.
Hydrochloric Acid	USP-NF or Ph. Eur.
Water for Injection	USP-NF Ph. Eur.[a]

Ph. Eur. = European Pharmacopoeia; *q.s.* = *quantum satis*; USP = United States Pharmacopeia; NF = National Formulary; BP = British Pharmacopeia; JP = Japanese Pharmacopeia

a Water for Injection meets USP and Ph. Eur. specifications at point of fill.

6 MITIGATION MEASURES FOR CLINICAL USE

Production of AVXS-101 takes place in closed premises according to standards for Biosafety Level 2 in the initial drug substance manufacturing steps. AVXS-101 manufacture takes place in Biosafety Level 1 after purification of drug substance. Any unused biological material (plasmid, bacterial, viral) and any materials that have been in contact with them are appropriately decontaminated and are collected for disposal using a contract service.

Handling of AVXS-101 vector product follows compliance standard for Biosafety Level 1 following the National Institute of Health Guidelines.

The product is most appropriately designated a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease.' Similar classifications of hazard have been assigned to wild type AAV according to the definitions of the World Health Organisation (WHO) Laboratory Biosafety Manual 2004, and in the US NIH Recombinant DNA guidelines 2016.

The vector-containing syringes are delivered to the treatment room and administered to the patient within eight (8) hours of removal from the refrigerator. All transfers are done in spill-proof containers to avoid spreading of the vector during transport. Individuals manipulating the vector will wear personal protective equipment in accordance with BSL level 1 requirements or as per local regulations or guidelines.

Workers shall change gloves and other related ppe (gowns) when contaminated; integrity is compromised; or when otherwise necessary. Gloves shall not be washed, and reuse of disposable gloves is prohibited. Dispose of used gloves with other contaminated waste. Hand washing protocols must be rigorously followed after glove removal.

All materials used for injection, including sterile drapes, needles, and syringes in contact with the vector are sealed in leak-proof primary and secondary containers. All waste are double bagged bearing the biohazard symbol and disposed of in a biohazard waste container in accordance with local requirements.

Spills will be soaked up with absorbent material and surfaces cleaned and disinfected with approved disinfectants (e.g. 10% bleach solution).

For infant patients who use diapers, to further reduce the potential for exposure to patient caregivers after subjects are discharged, the Marketing Authorisation Holder (MAH) will provide guidelines to family members and caregivers to practice good hand hygiene for a minimum of one month after ZOLGENSMA administration. This requires washing hands with soap regularly and using appropriate protective gloves if coming into direct contact with bodily fluids and waste. Disposable diapers should be disposed of in sealed disposable plastic trash bags and placed into the regular household waste stream after gene replacement therapy.

7 DETERMINATION OF THE OVERALL RISK OF THE GMO

Given that ZOLGENSMA is completely non-replicative and is not a known pathogen of any plant or animal species, and given that the wild type virus is naturally occurring in the environment, the risk to the environment from exposure to potentially contaminated material is considered to be minimal. ZOLGENSMA shedding is observed mainly in faecal material from treated patients based on data obtained from the AVXS-101-CL-101 study participants, as well as pre-clinical studies and observations from other rAAV clinical trials, where it rapidly declines to levels that can be practically considered as non-infectious, especially given the high multiplicity of infection (MOI) that is necessary for productive transduction (Ellis et al, 2013). Most importantly, the recombinant ZOLGENSMA vector is lacking all AAV9 viral genes and is incapable of generating a productive infection even in the presence of environmentally occurring helper viruses.

Due to the characteristics of the vector, the risk of integrational mutagenesis is negligible. In addition, the probability that genetic material would be transmitted from the product is very low due to the fact that there are no mobile elements involved and co-purification of possible genetic impurities (mainly kanamycin resistance gene) is controlled for every batch.

8 CONCLUSIONS

ZOLGENSMA is a recombinant adeno-associated viral vector based on a naturally occurring Adeno Associated Virus, which has been genetically engineered thus rendering it incompetent of replication. The modification has not changed the host range, or tropism of the vector and there are no environmental effects identified that could be caused by release of ZOLGENSMA. Shedding of the virus is minimal and infection of non-target human/animal/plant cells *in vivo* is expected to be low due to the high MOIs required for infection. AveXis Inc. has a validated method available to analyse the recombinant AAV product from patient and environmental samples and there are appropriate mitigation measures in place to minimise any release of the product to the environment. Considering the ultra-rare disease for which this drug product is aimed, the volumes of production and clinical use are very low. AveXis Inc. has analytical tests in place to control any impurities of environmental concern and there are instructions in place for proper waste handling and disinfection of any accidental release of the vector. Taken together, the environmental risks are adequately considered and where any possibilities for environmental effects are identified, thorough mitigation measures are applied to avoid any environmental impacts.

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