Protocol Number: AVXS-101-CL-102

Official Title: Phase I, Open-Label, Dose Comparison Study of AVXS-101 for Sitting but Non-ambulatory Patients with Spinal Muscular Atrophy

NCT Number: NCT03381729

Document Date: 17-Jun-2019

[redacted]

SIGNATURE PAGE

TITLE CL-102 Clinical Protocol **ID**: PRO-744

User	Role	Job Title	Version	Decision	Date Signed
[redacted]	Final Approvers	Vice President, Clinical Development	8.0	Accepted	18 Jun 2019 04:23 PM (America/Chicago)
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	Final Approvers	Senior Medical Director	8.0	Accepted	18 Jun 2019 01:42 PM (America/Chicago)
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[redacted]

AVXS-101
AVXS-101-CL-102

IND Number:	15699
Investigational Product:	AVXS-101
Protocol Title:	Phase I, Open-Label, Dose Comparison Study of AVXS-101 for Sitting but Non-Ambulatory Patients with Spinal Muscular Atrophy
Indication Studied:	Spinal Muscular Atrophy
Sponsor Address:	AveXis, Inc. 2275 Half Day Road Bannockburn, IL 60015
Protocol Version / Date:	Version 8, Amendment 7 / 17 June 2019

The study will be completed according to the guidelines of Good Clinical Practice. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial patients are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

Confidentiality Statement

1. ADMINISTRATIVE INFORMATION

1.1. Approval

This study will be conducted with the highest respect for the individual patients in accordance with the requirements of this clinical study protocol and in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki
- The International Council for Harmonisation, Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations

SIGNATURES: (may be applied electronically and will therefore be maintained in the electronic system):

[redacted]
Vice President of Clinical Development
AveXis, Inc.
Date (ddMmmyyyy)

[redacted]
Sr. Vice President, Regulatory Affairs
AveXis, Inc.
Date (ddMmmyyyy)

[redacted]
Senior Medical Director, Clinical Development
AveXis, Inc.
Date (ddMmmyyyy)

[redacted]
Senior Director of Clinical Operations
AveXis, Inc.
Date (ddMmmyyyy)

[redacted]
Interim Head of Biostatistics
AveXis, Inc.
Date (ddMmmyyyy)

[redacted]
Vice President, Head of Global Patient Safety
AveXis, Inc.
Date (ddMmmyyyy)

1.2. Summary of Changes

Please see Appendix 3.

1.3. Investigator's Agreement

I have received and read the Investigator's Brochure for AVXS-101. I have read the AVXS-101-CL-102 protocol and agree to conduct the study in accordance with the relevant current protocol(s). I agree to maintain the confidentiality of all information received or developed in connection with this protocol. I agree to personally conduct or supervise the investigation(s). I also agree to promptly report to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB/IEC approval, except where necessary to eliminate apparent immediate hazards to human subjects. I agree to protect the safety, rights, privacy, and well-being of study participants. I agree to comply with:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Council for Harmonization, Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6 (R2).
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations including but not limited to Informed Consent 21 CFR Part 56, Institutional Review Board Review in 21 CFR Part 56, Adverse Event Reporting as defined in Section 13.4 and in 21 CFR 312.64, Adequate/accurate and accessible records in accordance with 21CFR 312.62 and 312.68.
- Terms outlined in the study site agreement.
- Responsibilities of the Investigator (per regulatory guidelines and applicable regulations) I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in this protocol.

Confidentiality Statement

The confidential information in this document is provided to you as a Principal Investigator or Consultant for review by you, your staff, and the applicable Institutional Review Board/Independent Ethics Committee. Your acceptance of this document constitutes agreement that you will not disclose the information contained herein to others without written authorization from the Sponsor.

_____
Printed Name of Investigator

_____ _____
Signature of Investigator Date (ddMmmyyyy)

1.4. Key Contact Information

Role in Study	Contact information
Responsible Physician	[redacted] Medical Director, Clinical Development, [redacted]
Serious Adverse Event Reporting	[redacted]

Additional study contact information is provided in the Study Contact List.

2. SYNOPSIS

Name of Sponsor/Company: AveXis, Inc.	
Name of Investigational Product: AVXS-101	
Name of Active Ingredient: Survival Motor Neuron Gene by Self-Complementary Adeno-Associated Virus Serotype 9 (AAV9)	
Title of Study: Phase I, Open-Label, Dose Comparison Study of AVXS-101 for Sitting but Non-Ambulatory Patients with Spinal Muscular Atrophy	
Study center(s): 11 US investigators	
Studied period (years): Estimated date first patient enrolled: Q4 2017 Estimated date last patient completed: Q2 2021	**Phase of development:** 1

Objectives:

Safety will be assessed independently for each age cohort:

Primary:

- To assess the safety and tolerability of intrathecal (IT) administration of AVXS-101 by the incidence and severity of adverse events (AEs)
- To determine the optimal dose of AVXS-101 that demonstrates acceptable safety with maximum preliminary efficacy administered by IT injection

Efficacy for patients ≥ 6 months and < 24 months at time of dosing:

Primary:

- Proportion of patients ≥ 6 months and < 24 months at time of dosing achieving the ability to stand without support for at least three seconds (Bayley© Scales of Infant and Toddler Development – Gross Motor Subset #40)

Secondary:

- Proportion of patients that achieve ability to walk without assistance defined as taking at least five steps independently displaying coordination and balance (Bayley Scales of Infant and Toddler Development – Gross Motor Subset #43)

[redacted]

Efficacy for patients ≥ 24 months and < 60 months at time of dosing:

Primary:

- Change in HFMSE from baseline among patients ≥ 24 months at time of dosing

Secondary:

- Proportion of patients that achieve ability to walk without assistance defined as taking at least five steps independently displaying coordination and balance (Bayley Scales of Infant Development – Gross Motor Subset #43)

[redacted]

Additionally, compelling, demonstrable, documented evidence of efficacy as determined by changes in development abilities as captured during videotaping sessions during site visits and/or provided by parent/legal guardian will be collected for all patients in both age groups.

Methodology:

This is a Phase 1, single-dose administration study of infants and children with a genetic diagnosis consistent with spinal muscular atrophy (SMA), bi-allelic deletion of survival motor neuron 1 gene *(SMN1)* and 3 copies of survival motor neuron 2 gene *(SMN2)* without the genetic modifier who are able to sit but cannot stand or walk at the time of study entry. Patients will receive AVXS-101 in a dose comparison safety study of two (or three) potential therapeutic doses. Patients will be stratified in two groups, those ≥6 months and < 24 months of age at time of dosing and those ≥ 24 months and < 60 months of age at time of dosing. At least 15 patients ≥6 months and < 24 months, and at least 12 patients ≥ 24 < 60 months will be enrolled.

The first cohort will enroll three patients (Cohort 1) ≥6 months and < 24 months of age who will receive administration of 6.0×10^{13} vg of AVXS-101 (Dose A). There will be at least a four week interval between the dosing of each patient within the cohort. AveXis, Inc. (AveXis) will confer with the Data Safety Monitoring Board (DSMB) on all Grade III or higher AEs within approximately 48 hours of awareness that are possibly, probably or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB during quarterly meetings; following enrollment of the three patients and based upon the available safety data a decision will be made whether to: a) stop due to toxicity, or b) proceed to Cohort 2 using Dose B.

Should the determination be made to advance to Dose B, three patients < 60 months of age will be enrolled (Cohort 2) and will receive administration of 1.2×10^{14} vg of AVXS-101 (Dose B). Again, there will be at least a four-week interval between dosing of the three patients within the cohort. Based on the available safety data from the three Cohort 2 patients and all of the Cohort 1 patients, the DSMB will decide and document during quarterly meetings whether further four-week intervals between patients dosing is necessary. AveXis will take this recommendation into consideration and will make the final determination whether to persist with four-week intervals between patients dosing going forward; the decision will be communicated to sites and Institutional Review Boards (IRBs) in a formal sponsor letter. AveXis will confer with the DSMB on all Grade III or higher AEs within approximately 48 hours of awareness that are possibly, probably or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB during quarterly meetings; following enrollment of the first six patients and based upon available safety data, a decision will be made whether to: a) stop due to toxicity, or b) continue to enroll an additional 21 patients until there are a total of 12 patients > 6 months and < 24 months and 12 patients ≥ 24 and < 60 months that have all received Dose B.

Based upon an ongoing assessment of safety and efficacy data from patients treated with the 1.2×10^{14} vg dose, an option for testing of a third dose (Dose C), will be considered. If, based on all available data, this is judged to be safe and necessary, three patients < 60 months of age will receive Dose C, 2.4×10^{14} vg administered IT. A meeting of the DSMB will be called to obtain a recommendation on the safety of escalating to a higher dose prior to proceeding. If a decision is made to proceed to testing a higher dose, there will again be a four-week interval between dosing of the first three patients receiving Dose C, as in Cohorts 1 and 2. Safety data will be reviewed by the DSMB during quarterly meetings. Following enrollment of the first three Dose C patients and based upon available safety data, the DSMB will be consulted and a decision will be made whether to: a) stop dosing Dose C due to safety concerns, or b) continue to enroll an additional 21 patients until there are a total of 12 patients > 6 months and < 24 months and 12 patients ≥ 24 and < 60 months that have received Dose C.

Patients from Cohort 3 will be followed for a total of 15 months post-dose. The primary analyses for efficacy will be assessed when all patients reach 12 months post-dose and the primary analyses for

safety will be assessed when the last patient of Cohort 3 reaches 15 months post-dose (and database lock will be performed after the last patient reaches 15 months post-dose).

Safety will be assessed through monitoring AE reports and concomitant medication usage, and by conducting physical examinations, vital sign assessments, cardiovascular evaluations, and laboratory evaluations (chemistry, hematology, coagulation, urinalysis, immunology). Patients will be observed at the hospital for 48 hours post IT injection. Patients will return for follow-up visits according to the Schedule of Assessments (SoA).

An autopsy and tissue collection process will be in place for those patients who consent to autopsy/tissue collection for research purposes.

Upon study completion, study patients will be invited to participate in a long-term follow-up study conducted under a separate protocol.

Number of patients (planned): 27 (up to 51, if Dose C tested)

Diagnosis and main criteria for inclusion:
Patients with a genetic diagnosis consistent with SMA, bi-allelic deletion of *SMN1* and 3 copies of *SMN2* without the genetic modifier who demonstrate the ability to sit unassisted for 10 or more seconds but cannot stand or walk at the time of study entry.

Inclusion Criteria:

1. Patients ≥6 months and **≤ 60 months (1800 days) of age at time of dosing** following diagnostic confirmation during screening period by genotype who demonstrate the ability to sit unassisted for 10 or more seconds but cannot stand or walk
   - Diagnostic confirmation by genotype includes lab documentation of homozygous absence of *SMN1* exon 7; with exactly three copies of *SMN2*
2. Negative gene testing for *SMN2* gene modifier mutation (c.859G>C)
3. Onset of clinical signs and symptoms consistent with SMA at < 12 months of age
4. Able to sit independently and not standing or walking independently. Definition of sitting independently is defined by the World Health Organization Multicentre Growth Reference Study (WHO-MGRS) criteria of being able to sit up unsupported with head erect for at least 10 seconds. Child should not use arms or hands to balance body or support position (Wijnhoven 2004)
5. Meet age-appropriate institutional criteria for use of anesthesia and sedation, as determined necessary by the Investigator
6. Be up-to-date on childhood vaccines including palivizumab prophylaxis (also known as Synagis) to prevent respiratory syncytial virus (RSV) infections in accordance with the recommendations of the American Academy of Pediatrics (AAP 2009)
7. Parent(s)/legal guardian(s) willing and able to complete the informed consent process

Exclusion Criteria:

1. Current or historical ability to stand or walk independently
2. Contraindications for spinal tap procedure or administration of IT therapy (e.g., spina bifida, meningitis, impairment, clotting abnormalities, or obstructive spinal hardware preventing effective access to cerebrospinal fluid [CSF] space) or presence of an implanted shunt for the drainage of CSF or an implanted central nervous system (CNS) catheter
3. Severe contractures as determined by designated Physical Therapist(s) at screening that interfere with either the ability to attain/demonstrate functional measures (e.g., standing, walking) or interferes with ability to receive IT dosing
4. Severe scoliosis (defined as ≥ 50° curvature of spine) evident on X-ray examination
5. Previous, planned or expected scoliosis repair surgery/procedure within 1 year of dose administration

6. Use of invasive ventilatory support (tracheotomy with positive pressure) or pulse oximetry < 95% saturation at screening while the patient is awake, or for high altitudes > 1000 m, oxygen saturation < 92% while the patient is awake
   - Pulse oximetry saturation must not decrease ≥ four percentage points between screening and highest value on day of dosing
7. Use or requirement of non-invasive ventilatory support for 12 or more hours daily over the two weeks prior to dosing
8. Medical necessity for a gastric feeding tube, where the majority of feedings are given by non-oral methods (i.e., nasogastric tube or nasojejunal tube) or patients whose weight-for-age falls below the 3rd percentile based on World Health Organization (WHO) Child Growth Standards (Onis 2006). Placement of a permanent gastrostomy prior to screening is not an exclusion.
9. Active viral infection (includes human immunodeficiency virus (HIV) or serology positive for hepatitis B or C, or Zika virus)
10. Serious non-respiratory tract illness requiring systemic treatment and/or hospitalization within two weeks prior to study entry
11. Respiratory infection requiring medical attention, medical intervention or increase in supportive care of any manner within four weeks prior to study entry
12. Severe non-pulmonary/respiratory tract infection (e.g., pyelonephritis or meningitis) within four weeks before study dosing or concomitant illness that in the opinion of the Principal Investigator (PI) creates unnecessary risks for gene transfer such as:
    - Major renal or hepatic impairment
    - Known seizure disorder
    - Diabetes mellitus
    - Idiopathic hypocalciuria
    - Symptomatic cardiomyopathy
13. History of bacterial meningitis or brain or spinal cord disease, including tumors, or abnormalities by magnetic resonance imaging (MRI) or computerized tomography (CT) that would interfere with the lumbar puncture (LP) procedures or CSF circulation
14. Known allergy or hypersensitivity to prednisolone or other glucocorticosteroids or their excipients
15. Known allergy or hypersensitivity to iodine or iodine-containing products
16. Concomitant use of any of the following: drugs for treatment of myopathy or neuropathy, agents used to treat diabetes mellitus, or ongoing immunosuppressive therapy, plasmapheresis, immunomodulators such as adalimumab, or immunosuppressive therapy within 3 months of study dosing (e.g., corticosteroids, cyclosporine, tacrolimus, methotrexate, cyclophosphamide, intravenous (IV) immunoglobulin, rituximab)
17. Inability to withhold use of laxatives or diuretics in the 24 hours prior to dose administration
18. Anti-AAV9 antibody titers >1:50 as determined by enzyme-linked immunosorbent assay (ELISA) binding immunoassay
    - Should a potential patient demonstrate anti-AAV9 antibody titer > 1:50, he or she may receive retesting within 30 days of the screening period and will be eligible to participate if the anti-AAV9 antibody titer upon retesting is ≤ 1:50
19. Clinically significant abnormal laboratory values (gamma glutamyl transferase [GGT], alanine aminotransferase (ALT), and aspartate aminotransferase (AST), or total bilirubin > 2 × upper limit of normal (ULN), creatinine ≥ 1.0 mg/dL, hemoglobin [Hgb] < 8 or > 18 g/dL; white blood cell [WBC] > 20,000 per cmm) prior to gene replacement therapy. Patients with an elevated bilirubin level that is unequivocally the result of neonatal jaundice shall not be excluded.

20. Participation in recent SMA treatment clinical trial or receipt of an investigational or approved compound product or therapy received with the intent to treat SMA (e.g., valproic acid, nusinersen) at any time prior to screening for this study
    - Oral beta agonists must be discontinued 30 days prior to dosing.
    - Inhaled albuterol specifically prescribed for the purposes of respiratory (bronchodilator) management is acceptable and not a contraindication at any time prior to screening for this study
21. Expectation of major surgical procedures during the 1-year study assessment period (e.g., spinal surgery or tracheostomy)
22. Inability or unwillingness to comply with study procedures or inability to travel for repeat visits
23. Unwillingness to keep study results/observations confidential or to refrain from posting confidential study results/observations on social media sites
24. Refusal to sign consent form

Investigational product, dosage and mode of administration:
This is an open-label dose comparison study involving up to three AVXS-101 dosing cohorts of three patients each.
The AVXS-101 dosing cohorts are as follows:

- Cohort 1 (n=3) AVXS-101 6.0 × 1013 vg (Dose A)
- Cohort 2 (n=3) AVXS-101 1.2 × 1014 vg (Dose B)
- Cohort 3 (optional) (n=3) AVXS-101 2.4 × 1014 vg (Dose C)

AVXS-101 will be delivered to the IT injection procedure location premixed with 1.5 mL of an appropriate [redacted] approved and labeled for pediatric use for radiographic monitoring of the injection via lumbar IT injection. The total volume of AVXS-101 [redacted] will NOT exceed 8 mL. Sedation/anesthesia is required for all patients receiving AVXS-101. Method and medications will be at the discretion of the local anesthesiologist but should incorporate a sufficient degree of sedation or anxiolysis to ensure analgesia and lack of movement for the procedure and post-procedure Trendelenburg positioning placement (tilted head-down at 30° for 15 minutes). Patients will be placed in the Trendelenburg position, at 30°, for 15 minutes following administration of the AVXS-101 vector to enhance distribution to cervical and brain regions.

In an attempt to dampen the host immune response to the adeno-associated virus (AAV) derived therapy, patients will receive prophylactic prednisolone (glucocorticoid) (approximately 1 mg/kg/day) 24 hours prior to AVXS-101 dosing. Treatment will continue for approximately 30 days in accordance with specified guidelines for tapering.

Liver function testing should guide each step of the taper, and liver function tests should be checked prior to prednisolone discontinuation. If the GGT, AST, or ALT values are ≥ 2 × ULN, then the present dose of prednisolone will be adjusted as needed until the GGT, AST, and ALT values decrease below threshold, at which point the taper may continue. Liver function tests should also be checked approximately 2 weeks after the taper has concluded and prednisolone has been discontinued to evaluate for rebound elevation of GGT, AST or ALT levels. Variance from these recommendations will be at the discretion of the Investigator based on potential safety issues for each patient. If another glucocorticoid is used in place of prednisolone by the Investigator, similar considerations should be taken into account after 30 days and tapered as appropriate and at the discretion of the Investigator.

Based on the outcome of the safety analysis at the time of first applicable DSMB review of each cohort following enrollment of the third patient in each cohort, a decision will be made to stop the study due to toxicity, escalate to the next cohort or expand a cohort.

Duration of treatment: Single dose

Reference therapy, dosage and mode of administration: Not applicable

Criteria for evaluation:
Safety will be assessed independently for each age cohort:

- Incidence of Common Terminology Criteria for Adverse Events (CTCAE) Grade III or higher toxicity, treatment-emergent AEs
- Incidence of complications or AEs related to IT injection
- Clinically significant changes in laboratory values, physical exams, cardiovascular evaluations or vital signs
- Research Immunology Blood Labs: Serum antibody to AAV9 and SMN, interferon gamma (IFN-γ) Enzyme-Linked ImmunoSpot (ELISpot) to detect T-cell responses to AAV9 and SMN

Efficacy for patients ≥6 months and < 24 months at time of dosing:
Primary:

- Proportion of patients ≥6 months and < 24 months at time of dosing that achieve ability to stand alone (Bayley Scales of Infant and Toddler Development –Gross Motor Subset #40)

Secondary:

- Proportion of patients that achieve ability to walk without assistance defined as taking at least five steps independently displaying coordination and balance (Bayley Scales of Infant and Toddler Development –Gross Motor Subset #43)

[redacted]

Efficacy for patients ≥ 24 months and < 60 months at time of dosing:
Primary:

- Change from baseline in HFMSE among patients ≥ 24 months of age at time of dosing

Secondary:

- Proportion of patients that achieve ability to walk without assistance defined as taking at least five steps independently displaying coordination and balance (Bayley Scales of Infant and Toddler Development –Gross Motor Subset #43)

[redacted]

Additionally, compelling, demonstrable, documented evidence of efficacy as determined by changes in developmental abilities as captured during videotaping sessions during site visits and/or provided by parent/legal guardian will be collected for all patients in each age group.

Statistical Methods:

This is a Phase 1 trial with determination of optimal dose as primary objective, using both safety and efficacy measures on motor function assessments. Secondary outcomes include the achievement of milestones and change from baseline on HFMSE and fine and gross motor portions of the Bayley Scales of Infant and Toddler Development.

This study is designed to assess the impact of AVXS-101 on non-ambulatory young children with SMA who have 3 copies of *SMN2*. Data will be compared with patient-level data drawn from a widely (peer-reviewed) published natural history dataset collected by the Pediatric Neuromuscular Clinical Research (PNCR) network.

Patients will be stratified into 2 groups based upon age at dosing: 15 patients will be enrolled aged ≥6 months and < 24 months at dosing, and 12 patients will be enrolled who are aged 24 months to

< 60 months at the time of dosing. Up to an additional 24 patients, 12 aged ≥6 months and < 24 months at dosing, and 12 aged 24 months to < 60 months will be enrolled if the decision to escalate to Dose C is made.

The primary efficacy endpoint for the cohort of patients ≥6 months and < 24 months of age at time of dosing will be the proportion who achieve the ability to stand without support up to the 12-month study visit. The secondary efficacy endpoint will be the proportion of patients ≥6 months and < 24 months of age at the time of dosing who achieve the ability to walk without assistance up to the 12-month study visit. Based upon patient-level data available from the PNCR dataset, 14% of patients with *SMN2*=3 who meet the study criteria for patients ≥6 months and < 24 months of age achieved the ability to stand without support, and 10% achieved the ability to walk without assistance. We expect 85% of treated patients ≥6 months and < 24 months of age to achieve the ability to stand unassisted and 60% achieve the ability to walk without support. With this efficacy, a sample size of 12 patients would provide power of > 90% to detect a significant difference compared with the matched control cohort with $\alpha = 0.05$ using a two-sample 2-sided superiority Fisher exact test.

The primary efficacy endpoint for the cohort of patients between 24 months and < 60 months of age at time of dosing will be the change from baseline in HFMSE over 12 months. The secondary efficacy endpoint for patients between 24 months and < 60 months of age at the time of dosing will be the proportion of patients who achieve the ability to walk without assistance up to the 12-month study visit. We expect to observe an 8-point increase in HFMSE. With this efficacy, a sample size of 12 patients would provide power of > 90% to detect a significant difference with $\alpha = 0.05$ when compared to patient-level data available from the PNCR dataset using two-sample analysis of variance (ANOVA).

3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 1: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Definition
AAP	American Academy of Pediatrics
AAV	adeno-associated virus
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
BiPAP	Bilevel Positive Airway Pressure
BUN	blood urea nitrogen
C	celsius
CB	chicken-β-actin-hybrid
CDC	Center for Disease Control
CFR	Code of Federal Regulations
CK-MB	creatinine kinase-MB (isozyme MB)
cGMP	current Good Manufacturing Practice
CLIA	Clinical Laboratory Improvement Amendment
cmm	cubic millimeter
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
ddPCR	droplet digital polymerase chain reaction
dL	deciliter
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
eCRF	electronic Case Report Form
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked ImmunoSpot
FVB	friend virus b-type
GCP	Good Clinical Practice

Abbreviation or Specialist Term	Definition
GGT	gamma glutamyl transferase
GLP	Good Laboratory Practice
GFP	green fluorescent protein
HFMSE	Hammersmith Functional Motor Scale-Expanded
Hgb	hemoglobin
HIV	human immunodeficiency virus
ICD-10 code	International Statistical Classification of Diseases and Related Health Problems
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IFN-γ	interferon gamma
IRB	Institutional Review Board
IT	intrathecal(ly)
ITR	inverted terminal repeat
ITT	intent-to-treat
IV	intravenous(ly)
L3-L4, L4-L5	lumbar vertebrae 3, 4, 5
LP	lumbar puncture
mg	milligrams
mL	milliliter
MMRM	mixed model with repeat measurement
MRI	magnetic resonance imaging
NOAEL	no observable adverse effect level
OAE	other adverse event
PBMC	peripheral blood mononuclear cells
PI	principal investigator
PICU	Pediatric Intensive Care Unit
PNCR	Pediatric Neuromuscular Clinical Research
PPE	personal protective equipment
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RSV	respiratory syncytial virus
SAE	serious adverse event
SAP	Statistical Analysis Plan
scAAV	self-complimentary adeno-associated virus
scITR	self-complimentary inverted terminal repeat
SMA	spinal muscular atrophy

Abbreviation or Specialist Term	Definition
SMN	survival motor neuron
SMN1	survival motor neuron 1 gene
SMN2	survival motor neuron 2 gene
SoA	Schedule of Assessments
SUSAR	suspected unexpected serious adverse reaction
Syneos	contract research organization for this study
TMF	Trial Master File
µL	microliter
ULN	upper limit of normal
vg/kg	vector genome per kilogram
WBC	white blood cell
WHO	World Health Organization
WHO-MGRS	World Health Organization-Multicentre Growth Reference Study
WT or wt	wild type

5. INTRODUCTION

This is the first clinical gene therapy trial investigating intrathecal (IT) delivery for spinal muscular atrophy (SMA). The survival motor neuron (SMN) gene will be transferred using self-complementary adeno-associated virus (scAAV) Type 9 under control of the chicken-β-actin hybrid promoter. Pre-clinical studies have demonstrated survival of the SMN-Δ7 mouse model for SMA from a median of 15.5 days to over 1 year, following intravenous (IV) delivery to the facial vein. Additionally, an ongoing Phase 1 clinical study of SMA Type 1 patients has demonstrated improved muscle function and development milestone achievement, following IV delivery to a peripheral vein. This clinical trial is an open-label single injection ascending dose study in which AVXS-101 will be delivered one time through IT injection in non-ambulatory patients with SMA with 3 copies of survival motor neuron 2 gene (*SMN2)*.

5.1. Background and Preliminary Data

Spinal muscular atrophy is a neurogenetic disorder caused by a loss or mutation in the survival motor neuron 1 gene (*SMN1*) on chromosome 5q13, which leads to reduced SMN protein levels and a selective dysfunction of motor neurons. SMA is an autosomal recessive, early childhood disease with an incidence of 1: 10,000 live births (Sugarman 2012). SMA is the leading cause of infant mortality due to genetic diseases. Disease severity and clinical prognosis depends on the number of copies of *SMN2*. In its most common and severe form (Type 1), hypotonia and progressive weakness are recognized in the first few months of life, leading to diagnosis by 6 months of age and then death due to respiratory failure by age two. SMA Type 1 is the leading genetic cause of infant death. Motor neuron loss in SMA Type 1 is profound in the early postnatal period (or may even start in the pre-natal period), whereas motor neurons in Type 2 and 3 SMA patients adapt and compensate during development and persist into adult life. The findings from various neurophysiological and animal studies have shown an early loss of motor neurons in the embryonic and early postnatal periods (Swoboda 2005, Le 2011, Farrar 2012).

From a clinical perspective, these findings emphasized the importance of first targeting the SMA Type 1 group for gene transfer of *SMN2* in hopes of rescuing neurons at this critical stage. Our goal in continuing the development plan for AVXS-101 is to modify the SMA Type 2 phenotype, which will hopefully lead to a milder disease course and prolonged survival as seen in SMA Type 3 patients.

Therapeutic efforts in SMA have focused on the potential for small molecules to increase SMN levels. These include deacetylase inhibitors, such as, valproic acid, sodium butyrate, phenyl butyrate, and trichostatin A. These agents activate the *SMN2* promoter, resulting in increased full-length SMN protein in SMA animal models (Riessland 2010, Dayangac-Erden 2011).

However, clinical trials employing several of these agents, most notably phenyl butyrate, valproic acid, and hydroxyurea, have not resulted in clinical benefit (www.ClinicalTrials.gov and Darbar 2011). FDA recently approved Nusinersen, an antisense oligonucleotide 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 have shown some modest promise in improving motor function; however, the treatment must be administered indefinitely on a quarterly basis via IT injection, requires a lengthy induction period prior to effectiveness, and has safety considerations which require clinical monitoring. A dose escalation

trial of AVXS-101 injected IT will provide information for the potential gene transfer has in treating Type 2 and 3 SMA patients and will hopefully show promise for success in modifying the disease prognosis. This will be a dose comparison study that includes up to 51 Type 2/3 patients with 3 copies of *SMN2*.

5.2. Rationale for Gene Transfer to SMA Type 2/3 Patients

We have chosen SMA Type 2/3 as the target for this gene therapy study. We believe there is a strong rationale for this based on studies of the natural history of this disease. The classification of SMA is shown below (Table 2); Type 0 to Type 4 SMA is described. SMA is conventionally classified into four phenotypes on the basis of age of onset and highest motor function achieved, with an additional phenotype (Type 0) to describe the severe forms of antenatal-onset SMA (Mercuri 2012, Kolb 2011).

Table 2: Spinal Muscular Atrophy Classification

Type	Age at Symptom Onset		Maximum Motor Function	Life Expectancy	*SMN2* Copy No.
0	Fetal		Nil	Days – Weeks	1
1	< 6 Months	1A: B-2 Weeks 1B: < 3 months 1C: > 3 months	Never sits	< 2 years	1, **2**, 3
2	6 – 18 Months		Never walks	20 – 40 years	2, **3**, 4
3	1.5 – 10 Years	3A: < 3 Years 3B: > 3 Years	Walks, regression	Normal	3, **4**, 5
4	> 35 Years		Slow decline	Normal	4, 5

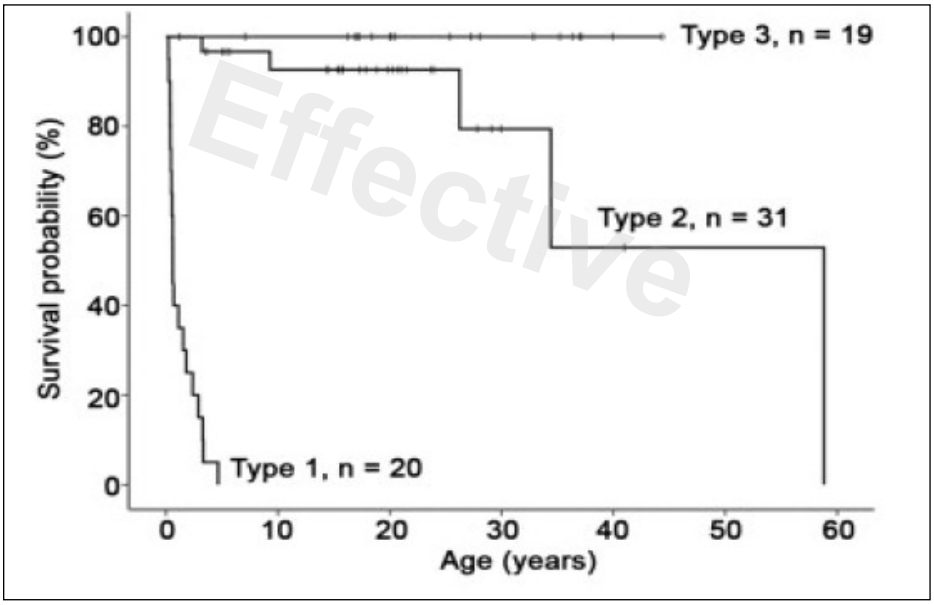
Source: Adapted from Kolb 2011.
SMN2 = survival motor neuron 2 gene
bold = predominant *SMN2* copy number that defines the SMA Type, the other copy numbers represent a small percentage of the designated SMA Type

SMA Type 1 patients by definition never attain independent sitting and have hypotonia within the first 6 months of life. SMA Type 1 is the leading genetic cause of infant death. In contrast, SMA Type 2 manifests within the first 18 months, and children afflicted with this condition are able to maintain sitting unassisted but never walk independently. SMA Type 3 patients attain the ability to walk unaided [Type 3a have onset <3yo; Type 3b have onset >3 yo]. SMA Type 4 is an adult onset disease. The genetic cause for SMA is well established and is intimately involved with one's prognosis. All forms of SMA are autosomal recessive in inheritance and are caused by deletions or mutations of the *SMN1*. Humans also carry a second nearly identical copy of the *SMN1* gene called *SMN2* (Lefebvre 1995). Both the *SMN1* and *SMN2* genes express SMN protein, however, the amount of functional full-length protein produced by *SMN2* is much less (by 10-15%) than that produced by *SMN1* (Lefebvre 1995, Lorson 1999, Monani 1999). Although *SMN2* cannot completely compensate for the loss of the *SMN1* gene, patients with milder forms of SMA generally have higher *SMN2* copy numbers (Lefebvre 1997, Park 2010). In a large early study by Feldkotter et al 2002 (Feldkotter 2002), 2 copies of *SMN2* was 97% predictive for developing SMA Type 1, 3 copies of *SMN2* was 83% predictive for developing SMA Type 2, and 4 copies of *SMN2* was 84% predictive of SMA Type 3. As these percentages

do not reflect the possible impact of modifier mutations such as that described by Prior et al 2009 (Prior 2009), they may understate the relationship between copy number (in the absence of a genetic modifier) and clinical phenotype (Feldkotter 2002). Among 113 patients with Type 1 SMA, 9 with one *SMN2* copy lived <11 months, 88/94 with two *SMN2* copies lived <21 months, and 8/10 with three *SMN2* copies lived 33–66 months. Even more refined data describing this relationship has been generated and has also influenced our choice of the study target group.

The severity of SMA Type 2 is demonstrated by prognosis as illustrated in Kaplan-Meier survival curves shown in Figure 1.

Figure 1: Kaplan-Meier survival curves and survival probabilities for SMA Type 1, 2, and 3



In Figure 1, the relative stability of the clinical course of SMA Types 2 and 3 is dramatically illustrated. Perhaps most importantly these findings show that outcome differences are related to the number of *SMN2* copies that enable motor neurons to adapt and compensate during the growth of the child and persist into adult life. This contrasts with SMA Type 1 where motor neuron loss is profound in the early postnatal period (or may even start in the pre-natal period, especially for SMA Type 1 patients presenting in first three months of life). The findings in Figure 1 confirm other pieces of evidence from neurophysiological studies and animal studies that also show early loss of motor neurons in the embryonic and early postnatal periods (Swoboda 2005, Le 2011, Farrar 2012). From a clinical trials perspective these findings emphasized the importance of first targeting SMA Type 1 for gene transfer of *SMN1* in hopes of rescuing neurons at this critical stage, as was the case for the ongoing Phase 1 clinical study with IV dosing. However, the delivery directly into the cerebrospinal fluid (CSF) via IT injection allows for reduction of the amount of viral vector by a factor of 10 with equal distribution and efficacy throughout the central nervous system (CNS) and reduced viral vector loads in major peripheral organs such as the liver, thereby further optimizing its safety profile (Meyer 2015, Duque 2015, Passini 2014). For these reasons, we are convinced that scAAV9 is the optimal

vehicle for delivering a functional full-length copy of *SMN* to SMA Type 2 and Type 3 patients, who are diagnosed at a later age and are thus potentially too great in size to receive a safe and effective weight-based IV dose. Ultimately, the goal is to modify the SMA Type 2 and 3 phenotypes leading to a milder course and improved functional development.

We also have good reason to believe that there are-few safety issues to be concerned about when targeting the SMA Type 2 and Type 3 groups for this clinical gene therapy trial. Overexpression of SMN has been shown to be well tolerated in both mice and non-human primates, and in human's high copy number of *SMN2* poses no risk (as seen in Type 2, 3, and 4 patients who have high *SMN2* copy number). This allows us to utilize robust, ubiquitous expression systems (like the chicken β-actin-hybrid [CB]-promoter) to ensure sustained, high-level SMN expression. Additionally, it is important to point out that recombinant scAAV can be employed for this trial because of the small size of the SMN gene. This enables efficient packaging and allows for efficient gene transfer with lower viral titers (a safety consideration), compared with prototypical single-stranded adeno-associated virus (AAV) vectors.

Our recent studies using scAAV9.CB.SMN show a robust postnatal rescue of SMA mice with correction of motor function, neuromuscular electrophysiology and survival after a one-time delivery of vector (Foust 2010). Intravenous scAAV9 is able to transduce neurons, muscle and vascular endothelium, all of which have been proposed as target cells for SMA treatment.

Taken altogether, our preclinical efficacy and toxicology studies in mice and non-human primates have increased our confidence that our Phase 1 trial will be safe and will have a high potential for success in treating this devastating disease.

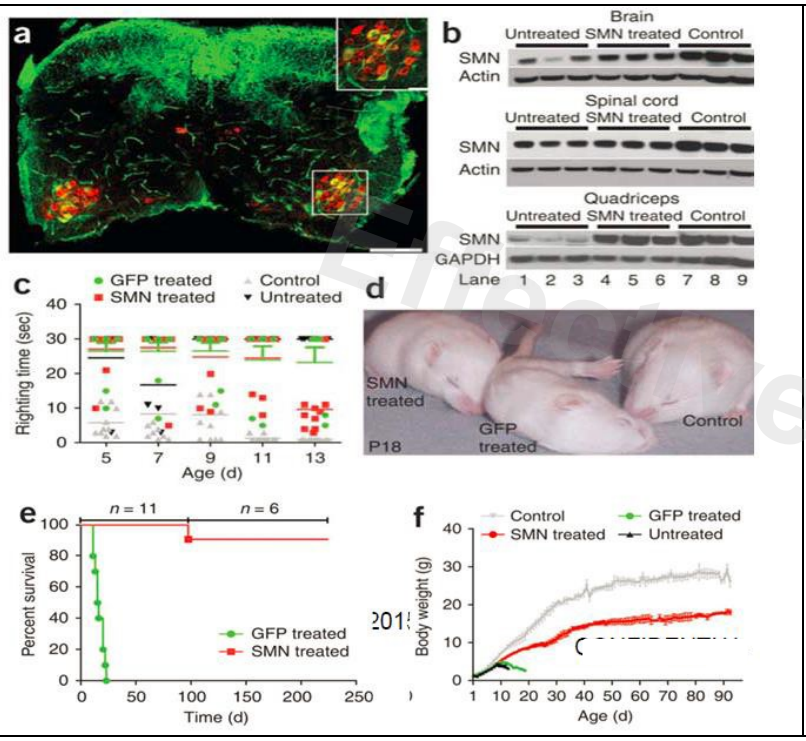
5.3. Non-Clinical Studies

A mouse model was developed by the Arthur Burghes Laboratory after a generation of multiple variants. It was found that the double transgenic, referred to as the SMN-Δ7 mouse, provided the most suitable model to study gene transfer (Butchbach 2007). Studies performed in the Kaspar laboratory have shown that injecting 5×10^{11} viral genomes of scAAV9.CB.SMN into the facial vein on Day 1 old mice rescues the SMN-Δ7 mouse model (Foust 2010). Figure 2 shows the results of these studies, including staining of transduced spinal motor neurons, SMN expression levels, righting ability, and weight and survival curves. Approximately 42 ± 2% of lumbar spinal motor neurons were transduced in scAAV9.CB.SMN treated mice. SMN levels were increased as well, in brain, spinal cord, and muscle of scAAV9.CB.SMN-treated animals, compared to untreated SMA mice (although lower than wild-type [WT] controls). SMA animals treated with either scAAV9.CB.SMN or scAAV9.CB.green fluorescent protein (GFP) on P1 were assessed for their righting ability and were compared to WT control mice and untreated mice. Wild type controls could right themselves quickly, whereas the SMN- and GFP-treated SMA animals showed difficulty at P5. However, by P13, 90% of SMN-treated animals could right themselves compared with 20% of GFP-treated controls and 0% of untreated SMA animals. At P18, SMN-treated animals were larger than GFP-treated animals, but smaller than WT controls. Locomotive ability of the SMN-treated mice was nearly identical to WT controls, as assayed by open field testing and wheel running.

Survival of SMN-treated SMA animals compared with GFP-treated SMA animals was significantly improved. No GFP-treated control animals survived past P22 and had a median life span of 15.5 days. The weights of GFP mice peaked at P10 and then precipitously declined until

death, while SMN mice showed a steady weight gain until around P40 with it stabilizing at 17 g (about half the weight of WT controls). The smaller size of corrected animals is likely related to the tropism and incomplete transduction of scAAV9, resulting in a 'chimeric' animal in which some cells were not transduced. Additionally, the smaller size suggests an embryonic role for SMN. Most remarkably, SMN-treated mice survived well past 250 days of age.

Figure 2: Study results, including staining of transduced spinal motor neurons, SMN expression levels, righting ability, and weight and survival curves



a Shows transduced motor neurons in lumbar spinal cord

b Western Blots of SMN expression in CNS and muscle

c Improved righting ability of SMN-treated- similar to WT controls by P13

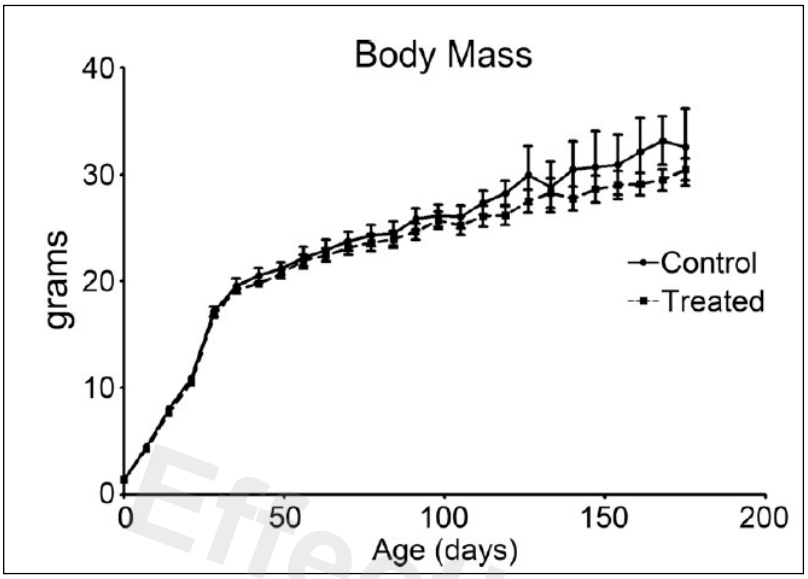
d SMN- treated are larger than GFP-treated at P18

e Survival of SMN-treated markedly improved compared to GFP- treated

f Body weight increased in SMN- treated vs GFP

Toxicology biodistribution studies were generated by the Kaspar laboratory. In the non-Good Laboratory Practice (GLP) studies, 24 mice and 4 non-human primates were injected, by way of vascular delivery, with scAAV9.CB.SMN. To assess toxicity and safety scAAV9.CB.SMN was injected into P1 WT friend virus b-type (FVB) mice with either vehicle (PBS) (3 males/6 females) or 3.3×10^{14} vg/kg of scAAV9.CB.SMN (6 males/9 females) via the facial temporal vein. This dose was previously shown to be most efficacious in the Δ7 mouse model of SMA (Foust 2010). P1 mice were used in anticipation of simulating potential clinical studies in infants, which is the planned population for the first-in-human clinical trial. All mice survived the injection procedure and the initial 24-hour observation period without any signs of distress or weight loss. Body mass was measured, and hands-on observations were performed weekly for the remainder of the study; neither revealed any difference between control and treated cohorts (Figure 3).

Figure 3: Body mass of treated and control mice showed no difference



At 60, 90, and 180 days post-injection, blood from the mice was collected for hematology studies and clinical chemistries assessment (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, creatinine, blood urea nitrogen [BUN], electrolytes, and creatinine kinase). All were normal except for one variant at the 90-day time point. This difference appeared to be due to a technical problem relating to the site of blood draw, which differed from that of all other mice. For histopathology, 13 mice were necropsied at 120 days post-injection and 8 mice at 180 days. All organs were normal; in particular there was no inflammation seen in any section from any organ (heart, liver, kidney, muscle, gonads, brain, lung, lymph nodes, and intestines).

In the safety study for the four male cynomolgus macaques, subjects were injected at 90 days of age to closely mimic the likely age of administration of treatment in SMA Type 1 infants. The scAAV9.CB.SMN vector was administered one time by catheterization of the saphenous vein with a dose of 6.7×10^{13}/kg, which corresponds to the lowest dose tested for which SMN-Δ7 mice showed a significant increase of survival. Animals were followed for six months until they were sacrificed at approximately 9 months of age. No adverse effects were seen, and all clinical chemistries were normal. T-cell immune response was tested using enzyme-linked ImmunoSpot (ELISpot) in peripheral blood mononuclear cells (PBMCs), and all were negative at 6 months post injection.

In these non-GLP studies, serum chemistry and hematology studies were unremarkable as was the histopathology assessment. The non-human primate subjects mounted appropriate immune responses to capsid (but not to transgene), with very high transgene expression persisting at 6 months post-injection. In conclusion, these studies provide strong evidence that systemically-delivered scAAV9.CB.SMN is safe and well tolerated, even at the high doses required for penetration of the blood-brain barrier (Foust 2010).

When newborn FVB mice were given a single IV injection of scAAV9.CB.SMN at levels up to 3.3×10^{14} vg/kg on Day 1, there was neither test article-related mortality nor evidence of toxicity

seen at time points up to 24 weeks after administration. Treatment-related decreases in mean body weight and mean body weight gain, as well as lower activated partial thromboplastin time values, were mild effects of treatment, but did not result in toxicity.

Activity of the scAAV9.CB.SMN was demonstrated by the bio distribution and the presence of a specific transgene ribonucleic acid (RNA) expression in brain and spinal cord, the main targeted therapeutic tissues. Low levels of antibodies to the AAV9 capsid were found after 12 and 24 weeks in males and females given 3.3×10^{14} vg/kg (Group 3). No alteration was observed in clinical pathology and histopathology analyses.

In these studies, scAAV9.CB.SMN IT administration to the CSF was safe and well tolerated in mice (through Week 12) and macaques (up to 14 months post injection). CSF delivery in mice likely reduced periphery exposure of scAAV9.CB.SMN and qualitative polymerase chain reaction (qPCR) results indicate transgene expression was higher in cervical and lumbar regions compared to the thoracic region. Monkeys maintained in the Trendelenburg position for 5 minutes at injection and were confirmed seronegative for anti-AAV9 antibodies prior to injection. All non-human primates were highly positive for AAV9 antibodies up to 6 months post injection. No cytotoxic T- lymphocyte response to either AAV9 capsid or SMN transgene was observed for 6 months post injection. No tissue degradation or reactive response in the brain or spinal cord was observed.

In pivotal GLP compliant 3-month mouse toxicology studies, the main target organs of toxicity were the heart and liver. Following IV infusion in the mouse, vector and transgene were widely distributed with the highest expression generally observed in heart and liver, and substantial expression in the brain and spinal cord. AVXS-101-related findings in the ventricles of the heart were comprised of dose-related inflammation, edema and fibrosis, and in the atrium, inflammation and thrombosis. Liver findings were comprised on hepatocellular hypertrophy, Kupffer cell activation, and scattered hepatocellular necrosis. A no observable adverse effect level (NOAEL) was not identified for AVXS-101-related heart and liver findings in the mouse, and the Maximum Tolerated Dose was defined as 1.5×10^{14} vg/kg, providing a safety margin of approximately 1.4-fold relative to the recommended therapeutic dose of 1.1×10^{14} vg/kg. The translatability of the observed findings in mice to primates is not known at this time.

These data support moving forward to clinical trials.

To determine whether CSF delivery can reduce the transduction of peripheral organs compared to the IV injections, a detailed bio distribution analysis was performed on the tissue of the nonhuman primates that were placed head down in the Trendelenburg position for either 5 or 10 minutes ($n = 5$). These animals were selected over the nonhuman primates that were not placed head down because the treatment highly improved distribution in the spinal cord and brain, favoring this approach for clinical trials. Two weeks post-injection, the cynomolgus macaques were sacrificed and various tissues were collected to perform detailed Deoxyribonucleic Acid (DNA) and RNA bio distribution analyses. scAAV9.CBA.GFP was lower in most peripheral tissues except spleen and liver compared to the high levels in brain and spinal cord. These findings are in line with previous reports from other groups (Dirren 2014, Gray 2013). In the skeletal muscles and the CNS, there is a strong correlation between DNA and RNA levels, while in soft tissues and glands, RNA levels are generally lower than expected for the viral genomes detected. In particular, testes, intestines, and spleen show a 1,000 times fewer RNA molecules than DNA. Despite the detection of AAV in peripheral organs (Dirren 2014, Gray 2013), there

was a significant decrease in the amount of vector detected peripherally compared to systemic injection. Additionally, similar observations were made when comparing mice that were injected either IV or intracerebroventricularly at P1 24 weeks post- treatment. Thus, CSF delivery is adding a significant potential safety component to future clinical trials with AVXS-101.

5.3.1. Trendelenburg Positioning Improves CSF Delivery

Dosing and efficacy of scAAV9-SMN was evaluated in SMA mice and non-human primates, delivered directly to the CSF via single injection. Widespread transgene expression was observed throughout the spinal cord in mice and nonhuman primates when using a 10 times lower dose compared to the IV application. In nonhuman primates, lower doses than in mice can be used for similar motor neuron targeting efficiency. The transduction efficacy was found to be further improved when subjects were kept in the Trendelenburg position to facilitate spreading of the vector (Meyer 2015). Tilting the animals significantly improved transduction in the thoracic and cervical region of the spinal cord, as demonstrated by immunofluorescence and quantification of GFP/ChAT double positive motor neurons. Tilting for 10 minutes was sufficient to increase motor neuron transduction to 55, 62, and 80% in the cervical, thoracic, and lumbar region respectively, which implies major benefits for patients according to the rescue observed in the mouse model. The motor neuron counts tightly correlated with GFP transcript quantification in each of the spinal cord segments.

5.4. Clinical Studies

First-in-human trial AVXS-101-CL-101 is a completed 2-year trial which evaluated the efficacy and safety of AVXS-101 in 15 SMA Type 1 patients with 2 copies of *SMN2*. All patients have received a single IV dose of AVXS-101 in 2 cohorts (Mendell 2017). After the End of Trial visit, patients were invited to participate in a long term follow up study conducted under a separate protocol.

Based on data obtained in Study AVXS 101-CL-101, the following conclusions can be made regarding the efficacy of AVXS-101: AVXS-101 administration had a positive effect on survival. All 15 patients were alive and free of permanent ventilation 24 months after dosing and all Cohort 2 patients had survived free of permanent ventilation, a statistically significant difference compared with the natural history rate of 8% reported by Finkel, 2014 (Finkel 2014).

5.5. Risks

A full understanding of all risks of AVXS-101 is not known at this time. Potential risks of AVXS-101 are discussed below and further details are provided in the AVXS-101 Investigator's Brochure. Please note, the risks discussed below are the result of observations with IV administration. The dose for IV therapy is 1- to 3-fold higher than for the IT therapy. Because the IT dose is lower than the IV dose and delivered directly to the CNS, the systemic exposure and associated risks are expected to be less.

Patients could experience an allergic response to AVXS-101. Patients could also develop an immune response to the AAV9 viral vector, which could prevent future use of gene transfers using this 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 2008). The relevance to humans with SMA is unknown.

Respiratory tract infections in neonates are very common in the general pediatric population (Paes 2001). In one study, an estimated 338 million new episodes of respiratory syncytial virus (RSV)-associated acute lower respiratory infections occurred worldwide in children younger than 5 years, with at least 34 million episodes necessitating hospital admission (Nair 2010). Respiratory syncytial virus is the primary cause of hospitalization for respiratory tract infection in young children (Hall 2001). It would not be unexpected that infants enrolled in the AVXS-101 gene replacement therapy trials might have similar incidences of respiratory infections due to these pathogens.

Patients must be clinically stable before AVXS-101 dosing. Clinical signs or symptoms of infection should not be evident at the time of AVXS-101 administration. Vaccinations, including palivizumab prophylaxis that can prevent RSV infections (Palivizumab SmPC 2019), are also recommended (Finkel 2018) and should be up to date. Added caution is advised regarding the timing of AVXS-101 administration in the presence of prodrome or resolving viral illness. In the event of a severe viral respiratory infection, the Investigator should be aware of the possibility of adrenal insufficiency in the presence of systemic immune response which may require longer glucocorticosteroid support at increasing doses to effectively manage the patient and prevent serious complications.

Some mice affected with SMA Type 1 that were treated with AVXS-101 experienced liver findings comprised on hepatocellular hypertrophy, Kupffer cell activation, and scattered hepatocellular necrosis.

Adverse events (AEs) of increased transaminases (ALT increased, AST increased, and transaminase increased) were reported as related to AVXS-101 in clinical studies. The underlying cause of the transaminase elevations is not known; though, may be related to an immune response to AAV9, as indicated by the response to prednisolone. Though these AEs can be serious, in most cases they were clinically asymptomatic, did not meet criteria for Hy's law, did not exhibit clinically relevant increases in bilirubin, and generally resolved with prednisolone treatment. A case of acute liver failure (serious unexpected serious adverse reaction [SUSAR]) was reported in the US Managed Access Program with AVXS-101. A 6-month-old male concurrently receiving nusinersen with elevations of AST and ALT of > 3 x upper limit of normal (ULN) before treatment with AVXS-101 developed acute liver failure approximately 51 days post AVXS-101 dosing. The patient recovered with additional steroid therapy.

Nonclinical cardiovascular toxicity findings that could potentially be relevant to the clinical use of AVXS-101 have been reported in 2 mouse toxicology studies of AVXS-101. Similar findings were reported in both studies. Findings in the ventricles of the heart were comprised of inflammation, edema and fibrosis. Primary findings in the atrium of the heart were thrombosis and inflammation. The underlying mechanism of these findings and the translatability of the observed findings in mice to primates are not known at this time.

The available clinical cardiovascular safety data have not provided evidence for a cardiovascular safety problem in humans. As of the last update to the Investigator's Brochure (v5), there have

been no cardiovascular AEs that have been judged to be related to AVXS-101 in the clinical studies.

A transient decrease in platelet counts has been observed with both IV and less frequently with IT administration. The majority of values remained above the lower limit of normal. Decreases were clinically asymptomatic and transient.

This risk can be effectively managed through monitoring platelet counts for 1 month following dosing, and appropriate prednisolone (or an equivalent corticosteroid in countries where prednisolone is not available) use.

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 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 wtAAV, also nonpathogenic, which has the ability to stably integrate into the host cell genome at a specific site (designated AAVS1) in the human chromosome 19 (Kotin 1990; Surosky). Since the AVXS-101 product uses AAV9 with all of the wtDNA removed from the capsids, except for the inverted terminal repeats (ITRs), 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 wtAAV2 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. For a review of the topic, see Srivastava and Carter, 2017 (Srivastava 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 wtAAV2. However, the rescue, replication, and packaging would stop 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 recombinant AAV (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 2001), except in one case in which very large doses of wtAAV and adenovirus were administered in a particular setting (Alfione 1996). Therefore, AAV9 interaction with other viruses to cause infection appears to be a minimal risk for AVXS-101.

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 caregivers 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.

Viral shedding is dependent on a variety of factors including the route of administration of the product, the tropism of the virus or bacteria, and the natural route of transmission and shedding of the parent virus or bacterium from which the product is derived. AveXis, Inc. (AveXis) collected saliva, urine, and stool samples at weekly timepoints through Day 30 and then monthly timepoints through Month 18 after gene transfer during the AVXS-101-CL-101 clinical study from five patients for viral shedding analysis. This analysis detects the number of genome copies by droplet digital polymerase chain reaction (ddPCR) in the applicable shed samples.

After IV administration, AVXS-101 is detectable in the shed samples from Day 1 post injection. All five patients analyzed were dosed with 2.0×10^{14} vg/kg. Concentrations of vector shed in saliva and urine are quite low and are below the limits of quantitation by ddPCR in the matrices within days post dose. Shedding of AVXS-101 was reported as a proportion of the initial concentration.

All five patients analyzed for viral shedding were dosed with the proposed therapeutic dose. AVXS-101 was detectable in shed samples post-infusion. 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, three patients for whom data were available showed a concentration of <1% of initial concentration in body at Day 14 post infusion, with concentrations declining approximately 4 logs (10,000-fold) over 30 days post infusion. Overall, AVXS-101 was primarily cleared from the body in stool and by Day 60 post infusion was below the limit of quantitation in stool. Shed AAV vectors have been previously shown not to be infectious in urine and saliva excreta (Favre 2001). Together, these data demonstrate rapid decline of shed vector quantities well below initial concentrations in patients treated with AVXS-101. Clearance of AVXS-101 is primarily via the feces, and the majority of the dose is cleared within 30 days of dose administration.

The results seen in the IV development program support further clinical investigation of the efficacy and safety of AVXS-101 in patients with SMA. Based on the current available data, safety events that appear to be associated with AVXS-101 consist of transient liver enzyme elevations, which have resolved following treatment with prednisolone. Taken together, results from the clinical and non-clinical studies support further clinical investigation of the efficacy and safety of AVXS-101 in patients with SMA.

6. TRIAL OBJECTIVES AND PURPOSE

6.1. Primary Objectives

The primary safety objective is to assess the safety and tolerability of IT administration of AVXS-101 by the incidence and severity of AEs while determining the optimal dose of AVXS-101 that demonstrates acceptable safety with maximum preliminary efficacy administered by IT injection. Safety and efficacy will be assessed independently for each age cohort.

6.1.1. Primary Objective: patients ≥ 6 months and < 24 months of age at time of dosing

The primary objective is to determine the proportion of patients ≥ 6 months and < 24 months of age at time of dosing achieving the ability to stand without support for at least three seconds (Bayley Scales of Infant and Toddler Development – Gross motor subset item # 40).

6.1.2. Primary Objective: patients ≥ 24 months and < 60 months of age at time of dosing

The primary objective is to determine the change from baseline in Hammersmith Functional Motor Scale- Expanded (HFMSE) for patients ≥ 24 and < 60 months of age at time of dosing.

6.2. Secondary Objective

The secondary efficacy objective for both age groups is to determine the:

- Proportion of patients that achieve ability to walk without assistance defined as taking at least five steps independently displaying coordination and balance (Bayley Scales of Infant and Toddler Development – Gross Motor subset item # 43).

[redacted]

7. INVESTIGATIONAL PLAN

7.1. Overall Study Design

The proposed clinical study is a Phase 1, open-label, single-dose administration study of infants and children with a genetic diagnosis consistent with SMA, bi-allelic deletion of *SMN1* and 3 copies of *SMN2* without the genetic modifier who are able to sit but cannot stand or walk at the time of study entry. Patients will receive AVXS-101 in a dose comparison safety study of up to three potential therapeutic doses as described below. Patients will be stratified in two groups, those ≥6 months and < 24 months of age at time of dosing and those ≥ 24 months and < 60 months of age at time of dosing. At least 15 patients ≥6 months and < 24 months will be enrolled and 12 patients ≥ 24 and < 60 months will be enrolled.

The first cohort will enroll three patients (Cohort 1) ≥6 months and < 24 months of age who will receive administration of 6.0×10^{13} vg of AVXS-101 (Dose A). There will be at least a 4-week interval between the dosing of each patient within the cohort. AveXis will confer with the Data Safety Monitoring Board/Data Monitoring Committee (DSMB/DMC) on all Grade III or higher AEs within approximately 48 hours of awareness that are possibly, probably, or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB/DMC during quarterly meetings; following enrollment of the first three patients and based upon the available safety data a decision will be made whether to: a) stop due to toxicity, or b) proceed to Cohort 2 using Dose B.

Should the determination be made to advance to Dose B, three patients < 60 months of age will be enrolled to receive administration of 1.2×10^{14} vg of AVXS-101 (Dose B). Again, there will be at least a 4-week interval between dosing of the three patients within the cohort. Based on the available safety data from the three Cohort 2 patients and all of the Cohort 1 patients, the DSMB/DMC may decide and document during quarterly meetings that further 4-week intervals between patients dosing is unnecessary. AveXis will take this recommendation into consideration and will make the final determination whether to persist with 4-week intervals between patients dosing going forward; the decision will be communicated to sites and Institutional Review Boards (IRBs) in a formal sponsor letter. AveXis will confer with the DSMB/DMC on all Grade III or higher AEs within approximately 48 hours that are possibly, probably, or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB/DMC during quarterly meetings; following enrollment of the first six patients and based upon available safety data, a decision will be made whether to a) stop due to toxicity, or b) continue to enroll an additional 21 patients until 12 patients ≥6 months and < 24 months and 12 patients ≥ 24 months and < 60 months have received Dose B.

Based upon an ongoing assessment of safety and efficacy data from patients treated with the 1.2×10^{14} vg dose, an option for testing of a third dose (Dose C), will be considered. If, based on all available data, this is judged to be necessary, three patients < 60 months of age will receive Dose C, 2.4×10^{14} vg administered IT. A meeting of the DSMB/DMC will be called to obtain agreement on the safety of escalating to a higher dose prior to proceeding. If agreement is obtained from the DSMB/DMC, there will again be a four-week interval between dosing of the first three patients receiving Dose C, as in Cohorts 1 and 2. Safety data will be reviewed by the DSMB/DMC during quarterly meetings; following enrollment of the first three Dose C patients and based upon available safety data, a decision will be made whether to: a) stop due to toxicity,

or b) continue to enroll an additional 21 patients until there are a total of 12 patients > 6 months and < 24 months and 12 patients ≥ 24 and < 60 months that have received Dose C.

Selection of the appropriate dose and justification for testing Dose C will be supported by ongoing safety and efficacy reviews of clinical findings from the patients receiving Dose B (1.2×10^{14} vg). Dose C will be 2.4×10^{14} vg delivered IT. Doses up to 1.1×10^{14} vg/kg have been safely administered systemically (IV) to children weighing up to 8.4 kg (total dose 9.24×10^{14} vg). In addition, in preclinical studies, the IT administration of scAAV9.CB.SMN was safe and well tolerated up to 14 months post injection in large non-human primates at a dose of 2×10^{13} vg/kg (AVXS-101 Investigator's Brochure).

The overall study design is summarized in Figure 4.

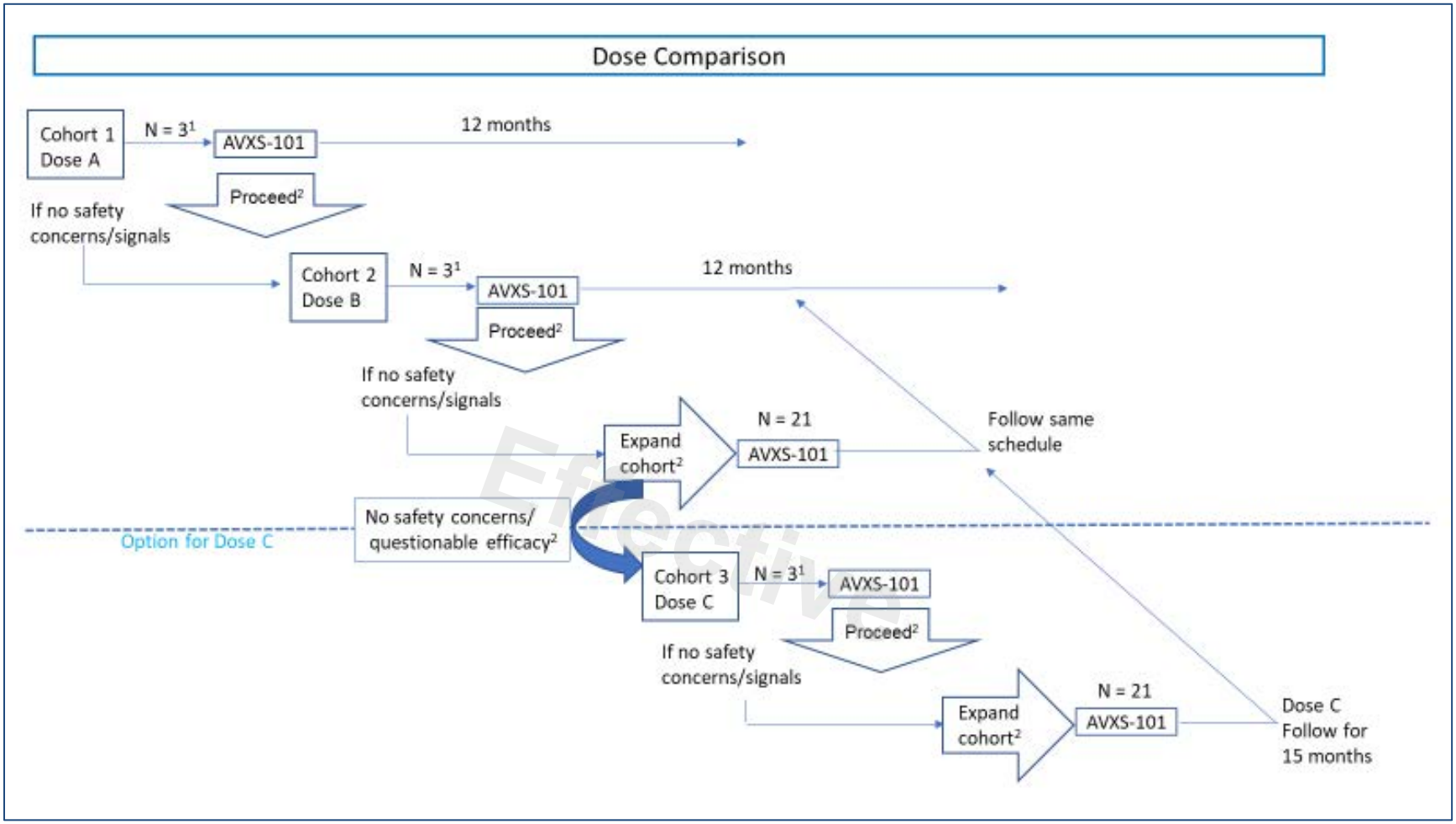
Safety will be assessed through monitoring AE reports and concomitant medication usage, and by conducting physical examinations, vital sign assessments, cardiovascular evaluations, and laboratory evaluations. Patients will be observed at the hospital for 48 hours post-IT injection. Patients will return for follow up according to the Schedule of Assessments (SoA) (Table 3).

Patients enrolled in Cohort 1 (Dose A) and Cohort 2 (Dose B) will complete 12 months of follow-up post IT administration. Patients enrolled in Cohort 3 (Dose C) will complete 15 months of follow-up post IT administration.

An autopsy and tissue collection process will be in place for those patients who consent to autopsy/tissue collection for research purposes.

Once patient completes the study, he or she will be invited to participate in a long-term follow-up study conducted under a separate protocol.

Figure 4: Study Design



1 Four week dosing between first three patients within each cohort to allow safety monitoring
2 Provided no dose related toxicities and DSMB/DMC determines acceptable to escalate/expand during quarterly meeting

Table 3: Schedule of Assessments[1]

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
Study Day	-60 to -2	-1	1	2-3	7	14	21	30	44	60	72	Monthly Through M12[1]	M15/ EOS	
Window					± 2							± 7		
Informed Consent	X													
Spinal X-ray	X													
Demographics/ Medical History	X		X	(X)	X	X	X	X		X		X	X	Medical history collected at baseline; review of systems conducted at each study visit. (X): Patients to remain inpatient 48 hours post-dose for observation and AE monitoring.
Physical Exam	(X)		X	X	X	X	X	X		X		X	X	Includes measuring head circumference. (X): Baseline procedure must be completed within 30 days of dosing.
Vitals/Weight/ Length/Height	(X)		X	X	X	X	X	X		X		X	X	Vital signs will include BP, respiratory rate, pulse, and axillary temperature. Vitals including BP, respiratory rate, pulse axillary temperature, pulse oximetry and HR will be monitored and recorded every 15 minutes (± 5 minutes) from dosing for four hours and every hour (± 15 minutes) for 24 hours following the AVXS-101 dosing procedure. (X): Baseline procedure must be completed within 30 days of dosing.
Pulse Oximetry	(X)		X	X	X	X	X	X		X		X	X	(X): Baseline procedure must be completed within 30 days of dosing.
Pulmonary Exam	(X)							X		X		X	X	

[1] Patients who reached the Month 12 visit prior to Protocol Amendment 7 had their EOS assessments conducted at Month 12.

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
Study Day	-60 to -2	-1	1	2-3	7	14	21	30	44	60	72	Monthly Through M12[1]	M15/ EOS	
Window					± 2							± 7		
12-Lead ECG	X		X	X								(X)	X	Irrespective of study schedule, after signing the updated informed consent, all patients enrolled prior to the Amendment 5 version of the protocol should have ECG, 24-hour Holter monitoring, and echocardiogram done at the next scheduled visit and then in conformity with the study SoA. (X): Months 3, 6, 9, and 12 visits.
12-Lead Holter Monitor	X	X	X	X				X		(X)		(X)	X	ECGs extracted from Holter by central reader in triplicate at the following time points: pre-dose, 2h, 4h, 6h, 8h, 12h, 24h, 36h, and 48h during the patient's inpatient stay. (X): Months 2, 3, 6, 9, and 12 visits.
Echocardiogram	X											(X)	X	Irrespective of study schedule, after signing the updated informed consent, all patients enrolled prior to the Amendment 5 version of the protocol should have echocardiogram done at the next scheduled visit and then in conformity with the study SoA. (X): Months 3, 6, 9, and 12 visits.
Capillary Blood Gas		X		(X)										(X): Laboratory samples collected on Day 2.
HFMS- Expanded (with video)	(X)							X				X	X	Will be conducted for patients ≥ 24 months of age; patients < 24 months of age at study entry will begin HFMS-E at such time that 24 months of age is reached. (X): Baseline procedure must be completed within 30 days of dosing.
Bayley III (with video)	(X)							X		X		X	X	Gross and fine motor sections will be performed at screening and monthly through 15 months. (X): Baseline procedure must be completed within 30 days of dosing.

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
Study Day	-60 to -2	-1	1	2-3	7	14	21	30	44	60	72	Monthly Through M12[1]	M15/ EOS	
Window					± 2							± 7		
Motor Milestone Development Survey (with video)	(X)							X		X		X	X	
Hematology / Chemistry	(X)	(X)*		(X)$	X	X	X	X	(X)†	X	(X)†	X	X	(X): Baseline procedure must be completed within 30 days of dosing. (X)*: Laboratory samples collected prior to dosing are to be processed locally. (X)$: Laboratory samples collected on Day 2. (X)† Liver function test (AST, ALT, total bilirubin, alkaline phosphatase, GGT) only.
Coagulation	(X)	(X)*		(X)$	X	X	X	X		X		X	X	
Urinalysis	(X)	(X)*		(X)$	X	X	X	X		X		X	X	
CK-MB	X				X			X		X		(X)	X	Troponin I will be measured instead of CK-MB in new patients who are screened and enrolled after amendment 5 (protocol version 6.0) goes into effect. Participants who have been screened and enrolled but who have not yet received gene replacement therapy (visit #2) at the time that amendment 5 (protocol version 6.0) goes into effect will have baseline troponin I testing prior to treatment with AVXS-101, and will have troponin I testing in place of CK-MB. CK-MB will be collected from all other participants. (X): Months 2, 6, 9, and 12 visits.
Troponin I	X				X			X		X		(X)	X	
Virus Serology	(X)													(X): Baseline procedure must be completed within 30 days of dosing.
Blood for diagnostic confirmation testing	X													To be performed by central laboratory.

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
Study Day	-60 to -2	-1	1	2-3	7	14	21	30	44	60	72	Monthly Through M12[1]	M15/ EOS	
Window					± 2							± 7		
Saliva, Urine, and Stool Samples (for viral shedding)		X		(X)	(X)	(X)		(X)						(X): Sites participating in the viral shedding sub-study will collect 24-hour full volume samples for urine and feces through 24 and 48 hours. All other sites will collect singular urine and feces samples within 24 hours and 48 hours of dosing. Full urine and feces samples will be collected for patients ≥ 48 months who are no longer in diapers.
Baseline Screening of Biological Mother (Anti-AAV9 Ab)	(X)													Serum sample collected at screening for anti-AAV9 antibodies. (X): Baseline procedure must be completed within 30 days of dosing. (X)*: Additional sampling may be performed at subsequent time points if ELISpot value(s) continue to be elevated, based on further discussion with the PI and Medical Monitor.
Immunology Labs (Anti-AAV9/SMN)	(X)				X	X	X	(X)						
Immunology Labs (IFN-γ T-cells)					X	X	X	(X)						(X): Additional sampling may be performed at subsequent time points if ELISpot value(s) continue to be elevated, based on further discussion with the PI and Medical Monitor.
Prophylactic Prednisolone		X	X	X	X	X	X	X	X	X	X			See Section 9.2.1. Daily dosing from 24 hours prior to scheduled AVXS-101 dose and continued as per protocol.
Study Product Administration [redacted]			X											
Photograph Injection Site			X		X	X	X	X						
Adverse Events	X	X	X	X	X	X	X	X		X		X	X	

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
Study Day	-60 to -2	-1	1	2-3	7	14	21	30	44	60	72	Monthly Through M12[1]	M15/ EOS	
Window					± 2							± 7		
Prior and Concomitant Medications	*To be collected from 2 weeks before study dosing until final study visit*													

AE = adverse event, ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK-MB = Creatinine kinase-MB (isozyme MB); ECG = electrocardiogram; ELISpot = enzyme-linked ImmunoSpot; GGT = gamma glutamyl transferase; IFN-γ = interferon gamma; PI = Principal Investigator; SoA = Schedule of Assessments;

7.2. Number of Patients

At least 27 patients will be enrolled; up to 51 patients may be enrolled if escalation to Dose C is determined necessary.

7.3. Treatment Assignment

This is an open-label comparative single-dose study. Treatment will be assigned in accord with the dose escalation schedule specified in Section 10.6.

7.4. Dose Adjustment Criteria

The study investigates a one-time IT injection of AVXS-101; therefore, no dose adjustments are possible.

7.5. Criteria for Study Termination

An independent DSMB/DMC and medical monitor will monitor safety data on a continual basis throughout the trial. The DSMB/DMC can recommend early termination of the trial for reasons of safety. Study enrollment may be halted when any patient experiences a Grade III, or higher AE toxicity that is unanticipated and possibly, probably or definitely related to the study product that presents with clinical symptoms and requires medical treatment. This will include any patient death, important clinical laboratory finding or any severe local complication in the injected area related to administration of the study agent. If after review by the DSMB/DMC the decision is made to continue, the study will proceed according to the dose escalation schedule.

The trial may be terminated for the following reasons:

- Development of unacceptable toxicity, defined as the occurrence of any unanticipated Common Terminology for Adverse Events (CTCAE) Grade 3 or higher AE/toxicity that is possibly, probably, or definitely related to gene replacement therapy, and is associated with clinical symptoms and/or requires medical treatment
- DSMB/DMC can recommend early termination of the study for safety reasons
- Study is terminated by sponsor
- Regulatory Authority recommendation

8. SELECTION AND WITHDRAWAL OF PATIENTS

Patients with a genetic diagnosis consistent with SMA, bi-allelic deletion of SMN1 and 3 copies of SMN2 without the genetic modifier who demonstrate the ability to sit unassisted for 10 or more seconds but cannot stand or walk at the time of study entry will be enrolled in this clinical trial. Patients will be of any racial, ethnic, or gender background. Enrollment will be staggered with at least four weeks between patient injections for all patients in Cohort 1 and at least the first three patients in Cohort 2 and Cohort 3.

8.1. Patient Inclusion Criteria

Patients must meet all of the following inclusion criteria:

1. Patients ≥6 months of age and ≤60 months (1800 days) of age at time of dosing following diagnostic confirmation during screening period by genotype who demonstrate the ability to sit unassisted for 10 or more seconds but cannot stand or walk
   - Diagnostic confirmation by genotype includes lab documentation of homozygous absence of *SMN1* exon 7; with exactly three copies of *SMN2*
2. Negative gene testing for *SMN2* gene modifier mutation (c.859G>C)
3. Onset of clinical signs and symptoms consistent with SMA at < 12 months of age
4. Able to sit independently and not standing or walking independently. Definition of sitting independently is defined by the World Health Organization- Multicentre Growth Reference Study (WHO-MGRS) criteria of being able to sit up unsupported with head erect for at least 10 seconds. Child should not use arms or hands to balance body or support position (Wijnhoven 2004)
5. Meet age-appropriate institutional criteria for use of anesthesia and sedation, as determined necessary by the Investigator
6. Be up-to-date on childhood vaccines, including palivizumab prophylaxis (also known as Synagis) to prevent RSV infections, in accordance with the recommendations of the American Academy of Pediatrics (AAP 2009)
7. Parent(s)/legal guardian(s) willing and able to complete the informed consent process

8.2. Patient Exclusion Criteria

Patients must not meet any of the following exclusion criteria:

1. Current or historical ability to stand or walk independently
2. Contraindications for spinal tap procedure or administration of IT therapy (e.g., spina bifida, meningitis, impairment, clotting abnormalities, or obstructive spinal hardware preventing effective access to CSF space) or presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter
3. Severe contractures as determined by designated Physical Therapist(s) at screening that interfere with either the ability to attain/demonstrate functional measures (e.g., standing, walking) or interferes with ability to receive IT dosing
4. Severe scoliosis (defined as ≥ 50° curvature of spine) evident on X-ray examination

5. Previous, planned or expected scoliosis repair surgery/procedure within 1 year of dose administration
6. Use of invasive ventilatory support (tracheotomy with positive pressure) or pulse oximetry < 95% saturation at screening while the patient is awake, or for high altitudes > 1000 m, oxygen saturation < 92% while the patient is awake
   - Pulse oximetry saturation must not decrease ≥ four percentage points between screening and highest value on day of dosing
7. Use or requirement of non-invasive ventilatory support for 12 or more hours daily in the two weeks prior to dosing
8. Medical necessity for a gastric feeding tube, where the majority of feedings are given by non- oral methods (i.e., nasogastric tube or nasojejunal tube) or patients whose weight-for-age falls below the 3rd percentile based on World Health Organization (WHO) Child Growth Standards (Onis 2006). Placement of a permanent gastrostomy prior to screening is not an exclusion.
9. Active viral infection (includes human immunodeficiency virus [HIV] or serology positive for hepatitis B or C, or Zika virus)
10. Serious non-respiratory tract illness requiring systemic treatment and/or hospitalization within two weeks prior to study entry
11. Respiratory infection requiring medical attention, medical intervention or increase in supportive care of any manner within four weeks prior to study entry
12. Severe non-pulmonary/respiratory tract infection (e.g., pyelonephritis or meningitis) within four weeks before study dosing or concomitant illness that in the opinion of the Principal Investigator creates unnecessary risks for gene transfer such as:
    - Major renal or hepatic impairment
    - Known seizure disorder
    - Diabetes mellitus
    - Idiopathic hypocalciuria
    - Symptomatic cardiomyopathy
13. History of bacterial meningitis or brain or spinal cord disease, including tumors, or abnormalities by magnetic resonance imaging (MRI) or computed tomography (CT) that would interfere with the lumbar puncture (LP) procedures or CSF circulation
14. Known allergy or hypersensitivity to prednisolone or other glucocorticosteroids or their excipients
15. Known allergy or hypersensitivity to iodine or iodine-containing products
16. Concomitant use of any of the following: drugs for treatment of myopathy or neuropathy, agents used to treat diabetes mellitus, or ongoing immunosuppressive therapy, plasmapheresis, immunomodulators such as adalimumab, or immunosuppressive therapy within 3 months of study dosing (e.g., corticosteroids, cyclosporine, tacrolimus, methotrexate, cyclophosphamide, IV immunoglobulin, rituximab)

17. Inability to withhold use of laxatives or diuretics in the 24 hours prior to dose administration
18. Anti-AAV9 antibody titers >1:50 as determined by enzyme-linked immunosorbent assay (ELISA) binding immunoassay
    - Should a potential patient demonstrate anti AAV9 antibody titer > 1:50, he or she may receive retesting within 30 days of the screening period and will be eligible to participate if the anti AAV9 antibody titer upon retesting is ≤ 1:50
19. Clinically significant abnormal laboratory values (gamma glutamyl transferase [GGT], ALT, and AST, or total bilirubin > 2 × ULN, creatinine ≥ 1.0 mg/dL, hemoglobin [Hgb] < 8 or > 18 g/dL; white blood cell [WBC] > 20,000 per cmm) prior to gene replacement therapy. Patients with an elevated bilirubin level that is unequivocally the result of neonatal jaundice shall not be excluded.
20. Participation in recent SMA treatment clinical trial or receipt of an investigational or approved compound product or therapy received with the intent to treat SMA (e.g., valproic acid, nusinersen) at any time prior to screening for this study
    - Oral beta agonists must be discontinued 30 days prior to dosing
    - Inhaled albuterol specifically prescribed for the purposes of respiratory (bronchodilator) management is acceptable and not a contraindication at any time prior to screening for this study
21. Expectation of major surgical procedures during the 1-year study assessment period (e.g., spinal surgery or tracheostomy)
22. Inability or unwillingness to comply with study procedures or inability to travel for repeat visits
23. Unwillingness to keep study results/observations confidential or to refrain from posting confidential study results/observations on social media sites
24. Refusal to sign consent form

8.3. Patient Withdrawal Criteria and Discontinuation

Patients meeting the following criteria will be withdrawn:

- Death
  - Autopsies will be requested of any patients, with the exception of untreated patients, that expire following participation in a gene transfer study
- Failure to comply with protocol-required visits or study procedures for 3 or more consecutive visits that are not rescheduled, unless due to hospitalization
- Parent(s)/legal guardian(s) withdraws consent
- Investigator discretion

Early termination procedures should be completed within 14 days for any patient who prematurely discontinues the study for any reason. Patients who terminate the study early for reasons other than death will be offered enrollment in a long-term follow-up study.

9. TREATMENT OF PATIENTS

9.1. Description of Study Product

The biological product is a non-replicating recombinant self-complementary adeno-associated virus serotype 9 (AAV9) containing the cDNA of the human SMN gene under the control of the cytomegalovirus enhancer/chicken-β-actin-hybrid promoter (CB). The 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 of which the transgene is transcribed, and the resulting protein is produced. Cells transduced with AVXS-101 (formerly scAAV9.CB.hSMN) express the human SMN protein.

Table 4: Investigational Product

	Investigational Product
Product Name	AVXS-101
Unit Dose	6.0×10^{13} vg (Dose A) 1.2×10^{14} vg (Dose B) 2.4×10^{14} vg (Dose C)
Route of Administration	Intrathecal Injection
Physical Description	Once thawed, AVXS-101 is a clear to slightly opaque, colorless to faint white solution, free of visible particulates

9.2. Prior and Concomitant Medications

Prior and concomitant medications will be captured in the electronic Case Report Form (eCRF) from two weeks prior to study dosing until the End of Study visit.

9.2.1. Prophylactic Administration of Prednisolone

An antigen specific T-cell response to the AAV vector was observed in the ongoing Phase 1 clinical study investigating AVXS-101 treatment via IV infusion. This is an expected response between 2-4 weeks following gene transfer. One possible consequence to such antigen specific T-cell response is clearance of the transduced cells and loss of transgene expression.

In an attempt to dampen the host immune response to the AAV based therapy, patients will receive prophylactic prednisolone (glucocorticoid) (approximately 1 mg/kg/day) 24 hours prior to AVXS-101 dosing. Treatment will continue for approximately 30 days in accord with the following treatment guideline:

- Until at least 30 days post-injection: 1 mg/kg/day
- Weeks 5 and 6 post-injection: 0.5 mg/kg/day
- Weeks 7 and 8 post-injection: 0.25 mg/kg/day
- Week 9 post-injection prednisolone discontinued

Liver function testing should guide each step of the taper, and liver function tests should be checked prior to prednisolone discontinuation. If the GGT, AST or ALT values are $\geq 2 \times$ ULN, then the present dose of prednisolone will be adjusted as needed until the GGT, AST, and ALT values decrease below threshold, at which point the taper may continue. Liver function tests

should also be checked approximately 2 weeks after the taper has concluded and prednisolone has been discontinued to evaluate for rebound elevation of GGT, AST or ALT levels. Variance from these recommendations will be at the discretion of the Investigator based on potential safety issues for each patient. If another glucocorticoid is used in place of prednisolone by the Investigator, similar considerations should be taken into account after 30 days and tapered as appropriate and at the discretion of the Investigator.

9.2.2. Prohibited Medications

Concomitant use of any of the following medications is prohibited:

- Drugs for treatment of myopathy or neuropathy
- Agents used to treat diabetes mellitus
- Therapy received with the intent to treat SMA (e.g., valproic acid, nusinersen).
  - Oral beta-agonists must be discontinued at least 30 days prior to gene therapy dosing.
  - Inhaled beta agonists may be used to treat respiratory complications of SMA provided such medications are dosed at clinically appropriate levels
- Ongoing immunosuppressive therapy, plasmapheresis, immunomodulators such as adalimumab, or immunosuppressive therapy within 3 months of starting the trial (e.g., corticosteroids, cyclosporine, tacrolimus, methotrexate, cyclophosphamide, IV immunoglobulin, rituximab)

Corticosteroid usage following completion of the prednisolone taper is permissible at the discretion of the managing physician as part of routine clinical management. The use of prednisone in such circumstances should be documented appropriately as a concomitant medication, and the event precipitating its usage should be appropriately documented as an AE.

Should the use of corticosteroids (aside from inhaled corticosteroids for bronchospasm) be considered as part of care during the course of the prednisolone taper, this medical management should be discussed with the AveXis medical monitor, who will be responsible for any indicated medication adjustments related to the taper.

9.2.3. Vaccinations

Where feasible, the vaccination schedule should be adjusted appropriately to accommodate prednisolone use. When avoiding vaccination while on steroids represents an undue delay or interruption of a vaccination schedule, vaccination should continue at the discretion and judgment of the treating physician given 1) the importance of maintaining childhood vaccination in this population and 2) the published literature that indicates that vaccination while on steroid doses 1 mg/kg/day or below is safe and effective (Mendell 2017; Kroger 2018).

9.3. Treatment Compliance

AVXS-101 will be administered as a one-time IT injection.

9.4. Randomization and Blinding

This is an open-label study. See Section 10.7 for a description of the dosing cohorts.

10. STUDY PRODUCT MATERIALS AND MANAGEMENT

AVXS-101 was manufactured in accordance with current Good Manufacturing Practice (cGMP).

10.1. Study Product

AVXS-101

10.2. Study Product Dose and Dose Justification

Patients will receive a one-time dose of AVXS-101 6.0×10^{13} vg, 1.2×10^{14} vg or a third dose of 2.4×10^{14} vg, if determined necessary via IT injection. The delivery directly into the CSF via IT injection allows for reduction of the amount of viral vector approximately by a factor of 10 with equal distribution and efficacy throughout the CNS, reducing viral vector loads and further optimizing safety (see Section 5.2) for additional details). Selection of the appropriate dose and justification for studying all dose escalations are further supported by ongoing safety and efficacy reviews of clinical findings from the patients receiving previous doses as described (see Section 7.1). The highest selected dose will be 2.4×10^{14} vg delivered IT. Doses up to 1.1×10^{14} vg/kg have been safely administered systemically (IV) to children weighing up to 8.4 kg (total dose 9.24×10^{14} vg). In addition, in preclinical studies, the IT administration of scAAV9.CB.SMN was safe and well tolerated up to 14 months post injection in large non-human primates at a dose of 2×10^{13} vg/kg (AVXS-101 Investigator's Brochure).

10.3. Study Product Packaging and Labeling

AVXS-101 kits are labeled with a specific kit number and batch/lot number assigned at the cGMP facility. The content of the labeling is in accordance with the local regulatory specifications and requirements.

10.4. Study Product Storage and Destruction

AVXS-101 kits will be stored in an appropriate, locked room under the responsibility of the Investigator or other authorized persons (e.g., pharmacists) in accordance with local regulations, policies, and procedures. Control of storage conditions, especially control of temperature (e.g., refrigerated/freezer storage) and information on in-use stability and instructions for handling prepared AVXS-101 should be managed in accordance with the Pharmacy Manual.

The vessel used for delivery of the vector should be resealed and processed for destruction in accord with applicable biohazardous waste guidelines for disposal.

10.5. Study Product Preparation

Preparation of AVXS-101 will be done aseptically under sterile conditions by a pharmacist.

AVXS-101 will be pre-mixed with an appropriate [redacted] approved and labeled for pediatric use for radiographic monitoring of the injection via lumbar IT injection. The total volume of AVXS-101 [redacted] will NOT exceed 8 mL, as outlined in the Pharmacy Manual.

The dose-delivery vessel will be delivered to the designated pediatric intensive care unit (PICU) patient room or other appropriate setting (e.g., interventional suite, operating room, dedicated

procedure room) with immediate access to acute critical care management. The vessel will be delivered in accord with the Pharmacy Manual.

10.6. Study Product Administration

Patients will receive AVXS-101 IT injection 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. After patients are admitted, vitals will be monitored immediately after dosing and every 15 (± 5) minutes for four hours and every hour (± 15 minutes) for 24 hours following the AVXS-101 dosing procedure.

Sites are instructed to use an atraumatic needle inserted with the bevel parallel to the dura fibers; this has been shown to considerably reduce damage to the dura and consequently decrease the risk for CSF leak after lumbar puncture including in children (Kiechl-Kohlendorfer 2003; Ebinger 2004).

Sedation/anesthesia is required for all patients receiving AVXS-101. Method and medications will be at the discretion of the local anesthesiologist but should incorporate a sufficient degree of sedation or anxiolysis to ensure analgesia and lack of movement for the procedure and post-procedure Trendelenburg positioning placement. Patients will be placed in the Trendelenburg position, tilted head-down at 30° for 15 minutes following administration of vector to enhance distribution to cervical and brain regions.

AVXS-101 will be administered by an Investigator [redacted] Patients will be placed in the lateral decubitus position and a catheter with stylet will be inserted by a lumbar puncture into the L3-L4 or L4-L5 interspinous space into the subarachnoid space. Subarachnoid cannulation will be confirmed with the flow of clear CSF from the catheter. Approximately 4 mL CSF will be removed for Dose A and Dose B, a volume of CSF closely approximating the volume of AVXS-101 plus contrast injected (up to 7 mL) will be removed for Dose C and disposed of as per institutional guidelines. AVXS-101 in the pre-mixed contrast solution will be injected directly into the subarachnoid space. Flushing of the injection needles with 0.5 mL saline will be allowed as per institutional standards/guidelines.

10.6.1. Post-Administration Procedures

Following AVXS-101 administration patients will return to a designated PICU bed, or other appropriate setting, with close monitoring of vital signs. Concomitant medications and all AEs/serious adverse events (SAEs) will also be monitored and documented following dosing procedures.

Patients will be kept in the PICU patient room or other appropriate setting (e.g., interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management for 48 hours for closer monitoring of mental status. 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, gloves, masks, glasses, and closed-toe shoes. Patients' families will be provided standardized, IRB-approved handouts regarding monitoring for mental status changes which includes

monitoring for severe headache, fever, irritability, neck pain or stiffness, light sensitivity, vomiting, and lethargy (tiredness). Patients may be discharged from the hospital when the following criteria are met:

- Afebrile
- Absence of hypersensitivity reactions
- Absence of meningismus
- Absence of abnormal laboratory values suggestive of possible CNS infection or complication

10.7. Dose Escalation

There will be a 4-week dosing interval between all patients within Cohort 1 to allow review of the safety analysis from six-time points (Days 1, 2, 7, 14, 21, 30) prior to dosing of the next patient.

AveXis will confer with the DSMB/DMC on all Grade III or higher AEs within approximately 48 hours of awareness that are possibly, probably or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB/DMC during quarterly meetings; following enrollment of the first three patients ≥6 months and < 24 months of age at the time of dosing and based upon the available safety data a decision will be made whether to: a) stop due to toxicity, or b) proceed to Cohort 2 using Dose B.

Should the determination be made to advance to Cohort 2 (Dose B), again there will be at least a 4-week interval between dosing of the first three patients < 60 months of age at the time of dosing within the cohort. Based on the available safety data from the first three Cohort 2 patients and all of the Cohort 1 patients, the DSMB/DMC may decide and document during quarterly meetings that further 4-week intervals between patients dosing is unnecessary. AveXis will take this recommendation into consideration and will make the final determination whether to persist with 4-week intervals between patients dosing going forward. The decision will be communicated to sites and IRBs in a formal sponsor letter. AveXis will confer with the DSMB/DMC on all Grade III or higher AEs within approximately 48 hours that are possibly, probably or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB/DMC during quarterly meetings; following enrollment of the first six patients and based upon available safety data, a decision will be made whether to a) stop due to toxicity or b) continue to enroll an additional 21 patients until 12 patients ≥6 months and < 24 months of age at time of dosing and 12 patients > 24 < 60 months of age at time of dosing have received Dose B.

Based upon an ongoing assessment of safety and efficacy data from patients treated with the 1.2×10^{14} vg dose, an option for testing of a third dose (Dose C), will be considered. If, based on all available data, this is judged to be safe and necessary, three patients < 60 months of age will receive Dose C, 2.4×10^{14} vg administered IT. A meeting of the DSMB/DMC will be called to obtain a recommendation on the safety of escalating to a higher dose prior to proceeding. If a decision is made to proceed to testing a higher dose, there will again be a four-week interval between dosing of the first three patients receiving Dose C, as in Cohorts 1 and 2. Safety data will be reviewed by the DSMB/DMC during quarterly meetings. Following enrollment of the

first three Dose C patients and based upon available safety data, the DSMB/DMC will be consulted and a decision will be made whether to: a) stop dosing Dose C due to safety concern, or b) continue to enroll an additional 21 patients until there are a total of 12 patients ≥6 months and < 24 months and 12 patients ≥ 24 and < 60 months that have received Dose C.

10.8. Study Product Accountability

This is an open-label study. Eligible patients will be assigned a unique patient identification number once informed consent is obtained and all eligibility criteria are met.

The pharmacist or designee will maintain accurate records of the quantities of AVXS-101 received, dispensed, and destroyed. The pharmacist or designee will document the date and time of delivery of the dose vessel to the dose procedure room as well as the time the used vessel was prepared for destruction as per the Pharmacy Manual.

10.9. Study Product Handling and Disposal

All materials used for injection, including sterile drapes, needles, and syringes in contact with the vector must be sealed in leak-proof containers. All waste must be sealed in bags bearing the biohazard symbol and disposed of in a biohazard waste container.

All transfers must be done in spill-proof containers. Individuals manipulating the vector will be required to wear PPE, such as gloves.

Any quality issue noticed with the receipt or use of AVXS-101 (e.g., deficiency in condition, appearance, pertaining to documentation, labeling, expiration date, etc.) should be promptly reported to AveXis in accord with procedures outlined in the Pharmacy Manual.

Under no circumstances will the Investigator supply AVXS-101 to a third party, allow AVXS-101 to be used other than as directed by this clinical trial protocol, or dispose of AVXS-101 in any other manner.

11. ASSESSMENT OF EFFICACY

11.1. Physical Assessments

11.1.1. Hammersmith Functional Motor Scale- Expanded

The HFMSE was devised for use in children with SMA Type 2 and Type 3, to give objective information on motor ability and clinical progression.

The HFMSE will be administered by a qualified clinical evaluator (e.g., licensed physical or occupational therapist, or national equivalent) in accord with Table 3 for all patients ≥ 24 months of age. Patients < 24 months of age at time of dosing will begin having HFMSE assessments at such time that 24 months of age is reached.

The HFMSE sessions will be videotaped in accord with the AVXS-101-CL-102 Videotaping Manual.

11.1.2. Bayley Scales of Infant and Toddler Development

Bayley Scales of Infant and Toddler Development© version 3 is a standardized, norm-referenced infant assessment and will be administered by a qualified clinical evaluator (e.g., licensed physical or occupational therapist, or national equivalent). The gross and fine motor subtests will be completed in accordance with Table 3. See AVXS-101-CL-102 Physical Assessments Manual.

Bayley Scales assessments will be videotaped in accordance with the AVXS-101-CL-102 Videotaping Manual.

11.2. Motor Milestone Development Survey

The achievement of significant motor milestones will be assessed by the qualified clinical evaluator (e.g., licensed physical or occupational therapist, or national equivalent) using a standard Motor Milestone Development Survey shown in Table 5 with definitions of each milestone driven by the Bayley Scales of Infant and Toddler Development (see AVXS-101-CL-102 Physical Assessments Manual). The clinical evaluator will record whether the patient has attained each of the milestones on the Motor Milestone Development Survey in accordance with Table 3. Once observed, a motor milestone is considered attained. The date of attainment of each motor milestone will be determined by the date of the visit in which the milestone is observed.

During the screening visit, the clinical evaluator will complete an assessment of baseline milestone achievement in accordance with Table 3; this assessment must be recorded on video and the findings must be documented in the source.

As the Bayley Scales do not necessarily require the child to repeat previously attained milestones, it is essential that each milestone be captured on video. Development milestone assessment sessions will be videotaped in accord with the AVXS-101-CL-102 Videotaping Manual.

Table 5: Motor Milestone Development Survey

Developmental Milestone- Bayley Scale Item Number	Performance Criteria
Head Control – Gross Motor Subtest Item #4	Child holds head erect for at least 3 seconds without support
Rolls from Back to Sides – Gross Motor Subtest Item #20	Child turns from back to both right and left sides
Sits Without Support – Gross Motor Subtest Item #26	Child sits alone without support for at least 30 seconds
Stands with Assistance - Gross Motor Subtest Item #33	Child supports own weight for at least 2 seconds
Crawls – Gross Motor Subtest Item #34	Child makes forward progress of at least 5 feet by crawling on hands and knees
Pulls to Stand – Gross Motor Subtest Item #35	Child raises self to standing position using chair or other convenient object for support
Walks with Assistance – Gross Motor Subtest Item #37	Child walks by making coordinated, alternated stepping movements
Stands Alone – Gross Motor Subtest Item #40	Child stands alone for at least 3 seconds after you release his or her hands
Walks Alone – Gross Motor Subtest Item #43	Child takes at least five steps independently, displaying coordination and balance

11.3. Video Evidence

Clinical assessments required at each study visit will be videotaped in an effort to produce compelling, demonstrable, documented evidence of efficacy, as determined by changes in functional abilities. AveXis will provide a secure and confidential upload process for transfer and storage of the videos from investigational sites to a contracted third-party vendor that will compile and arrange videos as per AveXis requirements. Any/all videos received at AveXis or the contracted vendor will be treated as confidential study data and will be the sole property of AveXis. AveXis and the contracted vendor will provide this secure, encrypted transfer and storage solution to properly protect the identities of patients/families on the videos, which may be shared with regulatory agencies and/or the medical community, and/or in appropriate venues to discuss the results of this clinical trial.

Videos will be provided to an independent, centralized reviewer for unbiased assessment of milestone achievement. The independent central reviewer will document whether the video displays evidence of having achieved each developmental milestone. The date of motor milestone achievement will be computed as the earliest date on which video evidence demonstrates the achievement of the specified developmental milestone.

Additionally, the parent(s)/legal guardian(s) may submit additional videos demonstrating achievement of developmental milestones at any time during the trial. These videos will be handled in the same manner in which the trial-derived videos are handled.

12. ASSESSMENT OF SAFETY

12.1. Safety Parameters

The primary outcome for this clinical trial is safety. Study discontinuation rules are based on the development of unacceptable toxicity, defined as the occurrence of any one Grade III or higher treatment-related toxicity.

12.1.1. Dose Limiting Toxicity

Dose limiting toxicity is defined as any grade III AE, according to the CTCAE version 4.03 that is possibly, probably or definitely related to the study agent. The CTCAE version 4.03 classifications are outlined in Table 6.

Table 6: Common Terminology Criteria for Adverse Events

Grade	Definition
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL.[a]
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting selfcare ADL.[b]
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE.

Source: Common Terminology Criteria for Adverse Events (version 4.03)
[a] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[b] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Study enrollment will be interrupted by the Investigators when any patient experiences a Grade III, or higher AE toxicity that is unanticipated and possibly, probably, or definitely related to the study product. The event will then be reviewed by the DSMB/DMC and an evaluation will be made as to whether the trial should be terminated early following the discontinuation rules.

Dose limiting toxicities must be immediately reported as per study safety reporting guidelines to ensure timely escalation to the DSMB/DMC.

12.1.2. Discontinuation Rules

An independent DSMB/DMC and medical monitor will monitor safety data on a continual basis throughout the trial. The DSMB/DMC can recommend early termination of the trial for reasons of safety. Study enrollment will be halted by the Investigators when any patient experiences a Grade III, or higher AE toxicity that is unanticipated and possibly, probably, or definitely related to the study product that presents with clinical symptoms and requires medical treatment. This will include any patient death, important clinical laboratory finding, or any severe local complication in the injected area related to administration of the study agent. If after review by

the DSMB/DMC the decision is made to continue, the study will proceed according to Section 7.1 of this protocol.

12.2. Demographic/Medical History

Patient demographics and medical history information will be collected at baseline and captured in the eCRF. Medical history throughout the study will be collected as a Review of Systems at each visit.

Medical History information will include:

- Familial history of SMA including affected siblings or parent carriers;
- Gestational age at birth;
- Length/height/head circumference at birth;
- Hospitalization information from time of birth including number, duration, and reason for hospitalizations including International Statistical Classification of Diseases and Related Health Problems (ICD-10) codes if available;
- Historical ventilatory support, if any;
- Historical feeding support, if any

12.3. Vital Signs

Vital signs will include blood pressure, respiratory rate, pulse, and axillary temperature within 30 days of dosing and at the time points specified in Table 3.

Vitals including pulse oximetry and heart rate will be continuously monitored and recorded by a team member during the injection.

After patients are admitted, vitals including blood pressure, respiratory rate, pulse axillary temperature, pulse oximetry, and heart rate will be monitored and recorded every 15 minutes (± 5 minutes) after dosing for four hours and every hour (± 15 minutes) for 24 hours following the AVXS-101 dosing procedure.

12.4. Weight and Length/Height

Weight and length and/or height, as appropriate, will be measured as per the time points specified in Table 3.

12.5. Physical Examination

Physical examination will include review of the following systems: head, eyes, ears, nose and throat, lungs/thorax, cardiovascular, abdomen, musculoskeletal, neurologic, dermatologic, lymphatic, and genitourinary.

The head circumference shall be measured with each physical examination. To measure head circumference, the examiner should securely wrap a flexible measuring tape around the circumference of the head, above the eyebrows over the broadest part of the forehead, above the

ears, and over the most prominent part of the occiput. The measurement should be taken 3 times, and the largest measurement should be recorded to an accuracy of 0.1 centimeters.

Baseline physical examinations must be completed within 30 days of dosing, and in accord with the time points specified in Table 3.

12.6. Vaccination Recommendations

Patients are encouraged to follow all routinely scheduled immunizations as recommended by the Center for Disease Control (CDC) including palivizumab prophylaxis (also known as Synagis) to prevent RSV infections in accordance with the recommendations of the American Academy of Pediatrics (AAP 2009).

12.7. Electrocardiogram

A 12-lead electrocardiogram (ECG) will be performed as specified in the SoA (Table 3). For patients enrolled in the study prior to Amendment 5 and irrespective of study schedule, after signing an updated informed consent, all patients should have a 12-lead ECG at their next scheduled visit and then in conformity with the SoA (Table 3).

The ECG will be interpreted locally by a cardiologist or designee for immediate safety evaluation. The ECG tracings or ECG machine data will also be collected for centralized review and interpretation by a cardiologist.

A 12-Lead ECG will be performed (concurrent with Holter Monitor) as specified in the SoA (Table 3). Additional electrophysiological monitoring will be at the discretion of the Investigator as per local institutional guidelines.

12.8. 12-Lead Holter Monitor

Patients will have a 12-lead continuous Holter monitor attached 24 hours prior to dose administration on Day -1. The Holter monitor will remain through 48 hours (Day 3). Serial ECG data will be pulled in triplicate from the Holter monitor data at the following time points:

- pre-dose
- 2 hour
- 4 hour
- 6 hour
- 8 hour
- 12 hour
- 24 hour
- 36 hour
- 48 hour

Twenty-four-hour Holter monitoring will also be performed as specified in the SoA (Table 3). For patients enrolled in the study prior to Amendment 5 and irrespective of study schedule, after signing an updated informed consent, patients should have a 24-hour Holter monitor at their next scheduled visit and then in conformity with the SoA.

Holter monitors will be provided to study sites along with a dedicated laptop for uploading the data from the memory cards for centralized review and analysis by a cardiologist within 24 hours of data upload. AveXis will be notified of any safety concerns from the centralized review.

12.9. Echocardiogram

An echocardiogram will be performed as specified in the SoA (Table 3). For patients enrolled in the study prior to Amendment 5 and irrespective of study schedule, after signing an updated informed consent, all patients should have a transthoracic echocardiogram at their next scheduled visit and then in conformity with the SoA (Table 3).

The echocardiogram will be interpreted locally by a cardiologist or designee for immediate safety evaluation. The echocardiograms will also be collected for centralized review and interpretation by a cardiologist.

12.10. Spinal X-ray

A spinal X-ray will be performed at screening/baseline to rule out patients with severe scoliosis or those that would require major spinal surgical procedures during the 1-year study assessment period.

12.11. Pulmonary Exam

Pulmonary examinations will be performed by a pulmonologist (or appropriate individual as per standard institutional practice) at the time points specified in the SoA (Table 3). Prior to trial entry, a pulmonologist (or appropriate individual as per standard institutional practice) will review and document ventilator usage in the 2 weeks prior to screening.

Patients may be fitted with a non-invasive positive pressure ventilator (e.g., bilevel positive airway pressure [BiPAP]) at the discretion of the pulmonologist and/or Investigator. Non-invasive ventilatory support equipment will be provided by AveXis through a third-party vendor if not covered by the patient's insurance.

Should the patient require non-invasive ventilator support at any time during the trial, the equipment to be provided must be approved by AveXis in order to ensure ability to upload usage data.

Patients requiring non-invasive ventilatory support will be asked to bring the machine to each study visit such that the study staff can remove the storage device which captures actual usage data. The hours per day usage data for each day between visits will be extracted with software provided by the device manufacturer into a format that will be transferred/transcribed to the clinical database.

12.13. Photographs of Injection Site

Photographs will be taken of the injection site at the time points specified in Table 3 to monitor healing of the injection wound. The Day 1 injection site photograph will be performed prior to the start of gene replacement therapy injection. AveXis will provide a secure and confidential upload process for transfer and storage of the photographs from the investigative sites to a contracted third-party vendor that will compile and arrange photographs as per AveXis requirements. Any/all photographs received at AveXis or the contracted vendor will be treated as confidential study data and will be the sole property of AveXis. AveXis and the contracted vendor will provide this secure, encrypted transfer and storage solution to properly protect the identities of patients/families in the photographs, which may be shared with regulatory agencies, the medical community, and/or in appropriate venues to discuss the results of this clinical trial.

12.14. Laboratory Assessments

Biological samples will be collected throughout the trial at the time points specified in Table 3. Biological samples will be collected and shipped to a central laboratory. Samples for laboratory tests required on the day prior to dosing (Day -1) will be collected prior to dosing and will be processed locally by the site's Clinical Laboratory Improvement Amendment (CLIA) -certified local laboratory. In some cases, samples may be collected locally due to a need for immediate results or other safety or logistical concerns.

Table 7: Total Blood Volume

Visit	Tests[1, 3]	Total Volume (mL)[2]
Screening	Hematology, chemistry/CK-MB[3] or Troponin I, coagulation, virus serology, immunology sample (AAV9/SMN Ab only), diagnostic confirmation sample	19.3-19.6
Day -1	Hematology, chemistry, coagulation, capillary blood gas	6.0
Day 2	Hematology, chemistry, coagulation, capillary blood gas	6.0
Day 7	Hematology, chemistry/CK-MB or Troponin I, coagulation, immunology sample	10.0-12.3
Day 14	Hematology, chemistry, coagulation immunology sample	9.0-11.0
Day 21	Hematology, chemistry, coagulation immunology sample	9.0-11.0
Day 30	Hematology, chemistry/CK-MB or Troponin I, coagulation, immunology sample	11.0-12.3
Day 44	Chemistry	1.3
Day 60	Hematology, chemistry/CK-MB or Troponin I, coagulation	6.0-6.3
Day 72	Chemistry	1.3

Visit	Tests[1, 3]	Total Volume (mL)[2]
Month 3	Hematology, chemistry, coagulation	5
Month 4	Hematology, chemistry, coagulation	5
Month 5	Hematology, chemistry, coagulation	5
Month 6	Hematology, chemistry/CK-MB or Troponin I, coagulation	6.0-6.3
Month 7	Hematology, chemistry, coagulation	5
Month 8	Hematology, chemistry, coagulation	5
Month 9	Hematology, chemistry/CK-MB or Troponin I, coagulation	6.0-6.3
Month 10	Hematology, chemistry, coagulation	5
Month 11	Hematology, chemistry, coagulation	5
Month 12	Hematology, chemistry/CK-MB or Troponin I, coagulation	6.0-6.3
Last Study Visit (Month 15)[4]	Hematology, chemistry/CK-MB or Troponin I, coagulation	6.0-6.3
Total Volume for Study 1-Year Duration		**137.9-147.3**

Immunology = Serum antibody to AAV9 and SMN, IFN-γ ELISpot to detect T-cell responses to AAV9 and SMN

[1] Immunology sample requires 4-6 mL of whole blood

[2] Maximum total blood volume specified for each visit; in cases where tests can be combined, lower blood volumes may be sufficient. Positive serology samples at screening and elevated T-cell responses at Day 30 may require additional surveillance samples not reflected in the table.

[3] Troponin I will be measured instead of CK-MB in new patients who are screened and enrolled after amendment 5 (protocol version 6.0) goes into effect. Participants who have been screened and enrolled but who have not yet received gene replacement therapy (visit #2) at the time that amendment 5 (protocol version 6.0) goes into effect will have baseline troponin I testing prior to treatment with AVXS-101, and will have troponin I testing in place of CK-MB. CK-MB will be collected from all other participants.

[4] Only patients enrolled in Cohort 3 will have a month 15 visit. Patients enrolled in Cohorts 1 and 2 will complete the study or have EOS visit at Month 12

In a case where sufficient blood cannot be collected from a patient, blood will be used in the following priority order with the first having greatest priority and last having the least priority:

1. Safety blood labs: chemistry → hematology → coagulation → creatinine kinase isozyme-MB (CK-MB) or troponin
2. Interferon gamma (IFN-γ) ELISpot to detect T-cell responses
3. Serum antibody to AAV9 and SMN
4. Genetic re-confirmation testing

If there is not sufficient blood volume to include the genetic reconfirmation testing sample at the screening visit, the patient must return before the inpatient period. All patients must have genetic reconfirmation testing completed.

12.14.1. Hematology

Hematology analysis will include a CBC with differential and platelet count with smear. Samples will be collected and shipped in accord with the laboratory manual provided by the central laboratory as per the time points specified in Table 3.

Immediate/same-day hematology analyses required during in-patient dosing, as determined by the Investigator, will be performed as per investigational site standard procedures at the local laboratory.

12.14.2. Serum Chemistry

Samples will be collected and shipped in accord with the laboratory manual provided by the central laboratory as per the time points specified in Table 3.

Immediate/same-day chemistry analyses required during in-patient dosing, as determined by the Investigator, will be performed as per investigational site standard procedures at the local laboratory.

Chemistry analysis will include the following at all study visits:

- Serum GGT
- AST/ALT
- Serum total bilirubin
- Direct bilirubin
- Albumin
- Glucose
- Total creatine kinase
- Creatinine
- BUN
- Electrolytes
- Alkaline phosphatase

CK-MB or troponin I will be collected as specified in the SoA (Table 3). Troponin I will be measured instead of CK-MB in new patients who are screened and enrolled after amendment 5 (protocol version 6.0) goes into effect. Participants who have been screened and enrolled but who have not yet received gene replacement therapy at the time that amendment 5 (protocol version 6.0) goes into effect will have baseline troponin I testing prior to treatment with AVXS-101 and will have troponin I testing in place of CK-MB. CK-MB will be collected from all other participants.

Investigators will receive laboratory results from all study visits from the central laboratory (except Day -1).

12.14.3. Virus Serology

The administration of an AAV vector has the risk of causing immune-mediated hepatitis. For patients who have HIV or positive serology for hepatitis B or C or Zika virus, administration of AAV vector may represent an unreasonable risk; therefore, negative serology testing must be confirmed at screening, prior to treatment. These samples will be collected and shipped in accord with the laboratory manual provided by the central laboratory.

12.14.4. Coagulation Studies

Coagulation studies include prothrombin time, partial prothrombin time, and international normalized ratio will be collected in accordance with the laboratory manual provided by the central laboratory. Coagulation studies will be performed as per the timepoints specified in Table 3.

12.14.5. Urinalysis

Urine samples will be collected in accord with the laboratory manual provided by the central laboratory as per the time points specified in Table 3.

Day -1 and immediate/same-day urinalyses required during in-patient dosing, as determined by the Investigator, will be performed as per investigational site standard procedures at the local laboratory.

Urinalysis will include the following parameters:

- Color
- Clarity/turbidity
- pH
- Specific gravity
- Glucose
- Ketones
- Nitrites
- Leukocyte esterase
- Bilirubin
- Blood
- Protein
- Red Blood Cells
- WBCs
- Squamous epithelial cells
- Casts
- Crystals

- Bacteria
- Yeast

12.14.6. Capillary Blood Gas

Capillary blood gas will be completed as per the time points specified in Table 3. A puncture or small incision will be made with a lancet or similar device into the cutaneous layer of the patients' skin at a highly vascularized area (heel, finger, toe). To accelerate blood flow and reduce the difference between the arterial and venous gas pressures, the area will be warmed prior to the puncture. As the blood flows freely from the puncture site, the sample will be collected in a capillary tube.

12.14.7. Research Immunology Blood

12.14.7.1. ELISA: Anti-AAV9 Ab

Blood samples will be collected and shipped to the central laboratory in accord with the laboratory manual to test for serum antibodies to AAV9 as per the timepoints specified in Table 3.

12.14.7.2. ELISA: Anti-SMN Ab

Blood samples will be collected and shipped to the central laboratory in accord with the laboratory manual to test for serum antibodies to SMN as per the timepoints specified in Table 3.

12.14.7.3. IFN-γ ELISpot

Blood will be collected and shipped to the central laboratory in accord with the laboratory manual to perform IFN-γ ELISpot to detect T-cell responses to AAV9 and SMN as per the timepoints specified in Table 3.

12.14.8. Baseline Screening of Biological Mother

There is potential that the biological mother of the enrolled patient may have pre-existing antibodies to AAV9 that may be transferred to the patient via placental transfer in utero or theoretically through breast milk. Informed consent will be requested from the biological mother of the patient to screen the biological mother for circulating antibodies to AAV9. Once informed consent has been obtained, the biological mother will have her blood drawn from a peripheral vein and shipped to the central laboratory for screening of anti-AAV9 antibodies.

If AAV9 antibodies are identified, the Investigator should discuss with the biological mother whether to continue or to stop breastfeeding.

Patients consuming banked breast milk from donor sources that cannot be tested for anti-AAV9 antibodies must be transitioned to formula prior to participation.

12.14.9. Blood for Diagnostic Confirmation Testing

A blood sample will be collected during the screening visit and shipped to the central laboratory in accord with the laboratory manual for re-confirmation of *SMN1* deletions, *SMN2* copy

number, and absence of exon 7 gene modifier mutation (c.859G>C). This will be done to ensure consistency in diagnostic testing practices.

12.14.10. Saliva, Urine, and Stool Collection

Studies have shown that some vector can be excreted from the body for up to a few weeks after injection; this is called "viral shedding". Vector shedding can be found in the blood, urine, saliva, and stool for up to a week 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, IRB-approved 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.

Saliva, urine, and stool samples will be collected in accord with the laboratory manual for viral shedding studies in accord with Table 3. Patients at all sites ≥ 48 months of age who are no longer in diapers will provide full volume urine and full volume feces samples at the timepoints specified in the SoA (Table 3) for at least one void and one defecation. Samples will be prepared as per the laboratory manual, stored in a -80 freezer, and shipped to the central laboratory in accord with the laboratory manual.

A subset of patients at sites opting to participate in the viral shedding sub-study will have 24-hour total volume urine and fecal samples collected through 24 hour-post dose and 48 hours-post dose (to include all excretions in those time periods).

13. ADVERSE AND SERIOUS ADVERSE EVENTS

13.1. Definition of Adverse Events

13.1.1. Adverse Event

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including baseline or washout periods, even if no study treatment has been administered.

All AEs that occur after any patient has received treatment, during treatment or up through the last study visit, whether or not they are related to the study, must be recorded on forms provided by Syneos Health, formerly INC (a Contract Research Organization responsible for managing specific aspects of the study, as designated by AveXis).

All AEs will be classified in accordance with the CTCAE version 4.03 outlined in Table 6.

Study enrollment will be interrupted should any patient experience an unanticipated CTCAE Grade 3, or higher AE toxicity that is possibly, probably or definitely related to the product. The event will then be reviewed by the DSMB/DMC and an evaluation will be made as to whether the study should be terminated early following the discontinuation rules.

Unanticipated CTCAE Grade 3 or higher AEs that are possibly, probably or definitely related to gene replacement therapy must be reported within 24 hours to AveXis and/or designee to ensure timely escalation to the DSMB/DMC.

13.1.2. Serious Adverse Event

An SAE is an AE occurring during any study phase (i.e., baseline, treatment, washout, or follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following:

- Results in death
- It is immediately life-threatening
- It requires in-patient hospitalization or prolongation of existing hospitalization
- It results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect
- It is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above

All SAEs that occur after any patient has been enrolled, before treatment, during treatment, or up through the last study visit, whether or not they are related to the study, must be recorded on forms provided by Syneos Health.

13.1.3. Other Adverse Event

The following specific treatment-emergent AEs of special interest (AESIs), which may be searched using Standardized MedDRA queries, will be summarized:

- Elevated liver enzymes

Grade 3 or higher elevated liver enzyme events related to AVXS-101 must be collected and recorded on forms provided by the Contract Research Organization. These events should be reported within 24 hours of occurrence, whether or not they are deemed to be an SAE.

Other adverse events (OAEs) could be identified by the Drug Safety Physician and, if applicable, also by the Clinical Trial Team Physician during the evaluation of safety data for the Clinical Study Report. Examples **of AESIs** include hepatotoxicity, cardiac toxicity, and thrombocytopenia. Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the patient from the study, will be classified as OAEs. For each OAE, a narrative may be written and included in the Clinical Study Report.

13.2. Relationship to Study Product

An Investigator who is qualified in medicine must make the determination of relationship to the investigational product for each AE (unrelated, possibly related, probably related or definitely related). The Investigator should decide whether, in his/her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. If no valid reason exists for suggesting a relationship, then the AE should be classified as "unrelated." If there is any valid reason, even if undetermined, for suspecting a possible cause-and-effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered "related." If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable" the event will be considered to be related to the investigational product for the purposes of expedited regulatory reporting.

13.3. Recording Adverse Events

Adverse events spontaneously reported by the patient or family and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. However, abnormal values that constitute an SAE or lead to discontinuation of administration of study product must be reported and recorded as an AE. Information about AEs will be collected from the time of IT injection until the end of the study. Serious adverse event information will be collected from signing of consent form until the last study visit. The AE term should be reported in standard medical terminology when possible. For each AE, the Investigator will evaluate and report the onset (date and time, if start date during inpatient period), resolution (date and time, if start date during inpatient period), intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study.

Intensity will be assessed according to Table 6 in Section 13.1.1.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 13.1.2. An AE of severe intensity may not be considered serious.

13.4. Reporting Serious Adverse Events

All SAEs (related and unrelated) will be recorded from signing of consent form through the last study visit. Any SAEs considered possibly, probably, or definitely related to the investigational product and discovered by the Investigator at any time after the study should be reported. All SAEs must be reported to AveXis via Syneos Health within 24 hours of the first awareness of the event. The Investigator must complete, sign and date the SAE pages, verify the accuracy of the information recorded on the SAE pages with the corresponding source documents, and send a copy by fax or email to Syneos Health.

Additional follow-up information, if required or available, should all be faxed or emailed to Syneos Health within 24 hours of receipt and this should be completed on a follow-up SAE form, and placed with the original SAE information and kept with the appropriate section of the eCRF and/or study file.

AveXis is responsible for notifying the relevant regulatory authorities of certain events. It is the Principal Investigator's responsibility to notify the IRB/IEC of all SAEs that occur at his/her site, as per IRB/IEC reporting guidelines. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical trial. Each site is responsible for notifying its IRB/IEC of these additional SAEs.

13.5. Expedited Safety Reporting to Regulatory Authorities

All unexpected, serious, drug-related events which are fatal or life-threatening will be reported to relevant regulatory authorities in an expeditious manner. All other unexpected, serious, drug-related events will be reported to relevant regulatory authorities within 15 days.

14. STATISTICS

14.1. Statistical Methods

A Statistical Analysis Plan (SAP) will be developed that describes in detail the statistical methods to be used for analysis of this study and will be finalized prior to study enrollment.

14.2. General

Binary endpoints (e.g., standing without support) will be compared between AVXS-101 and natural history control groups using a 2-sided Fisher's Exact Test at level of 0.05. A 95% exact unconditional confidence interval for the true population difference in response rates will be computed.

Results involving continuous endpoints (e.g., change in HFMSE) will be compared between AVXS-101 and natural history control groups at 2-sided significance level α=0.05 using analysis of variance (ANOVA), or non-parametric Wilcoxon Rank Sum test, as appropriate.

All statistical tests will be conducted at a significance level of 0.05.

14.3. Analysis Populations

14.3.1. Full Analysis Set

The full analysis set consists of all patients who receive the AVXS-101 injection.

14.3.2. Intent-to-Treat

The Intent-to-Treat (ITT) Set will include all enrolled patients who receive AVXS-101 IT injection treatment. Patients will be analyzed according to the assigned dose. All efficacy analyses will be conducted using the ITT Set as the primary population, unless specified otherwise.

14.3.3. Enrolled Set

The Enrolled Set will include all patients enrolled (i.e., completed the informed consent process). Unless specified otherwise, this set will be used for patient listings and for summaries of patient disposition.

14.3.4. Efficacy Completers Analysis Set

The Efficacy Completers Analysis Set consists of all treated patients who complete 12 months following AVXS-101 dose procedure.

14.3.5. Safety Analysis Set

The safety analysis set consists of all patients who receive the AVXS-101 injection.

14.4. Sample Size Calculation

This is a Phase 1 trial with determination of optimal dose as primary objective, using both safety and efficacy measures on motor function assessments. Secondary outcomes include the

achievement of milestones and change from baseline on HFMSE and fine and gross motor portions of the Bayley Scales of Infant and Toddler Development.

This study is designed to assess the impact of AVXS-101 on non-ambulatory young children with SMA who have 3 copies of *SMN2*, with efficacy outcomes specifically assessing improvement in motor function from that expected in the natural history of non-ambulatory children with SMA. Data will be compared with patient-level data drawn from a widely (peer-reviewed) published natural history dataset collected by the Pediatric Neuromuscular Clinical Research (PNCR) network.

Although patients with SMA Type 2 and 3 are easily differentiated in a retrospective manner by best clinical function, given the clinical overlap between the two phenotypes it is difficult at the time of diagnosis to predict the ultimate clinical course for a given individual patient at the time of initial diagnosis. From the published literature from a large natural history study (Kaufman 2012), we believe that a significant majority of the patients eligible for this study will, in fact, have SMA Type 2. However, it is also expected based upon this literature that a small percentage of children eligible for this study (3 copies of *SMN2*, age of symptom onset less than 12 months, sitting but not standing or walking independently) will, in fact, be children who would ultimately walk independently as part of the normal course of their disease (and therefore would be ultimately re-classified as SMA Type 3). The study design, statistical considerations, and study size are based upon this presumption.

Patients will be stratified into 2 groups based upon age at dosing. Between Dose A and Dose B 15 patients will be enrolled aged ≥6 months and < 24 months at dosing, and 12 patients will be enrolled who are aged 24 months to < 60 months at the time of dosing. Up to an additional 24 patients may be enrolled and receive a higher dose (Dose C) if deemed appropriate and necessary by AveXis and approved by the DSMB/DMC (see Section 15).

The primary efficacy endpoint for the group of patients ≥6 months and < 24 months of age at time of dosing will be the proportion of treated patients who achieve the ability to stand without support up to the 12-month study visit. Twelve children aged ≥6 months and < 24 months will be enrolled who receive Dose B. The proportion of patients developing the ability to stand without support will be compared with a cohort of eligibility-matched patients from the PNCR natural history study of SMA. Based upon a review of eligibility-matched patients from the PNCR, 14% of patients who meet the study criteria for patients ≥6 months and < 24 months of age achieved the ability to stand without support, and 10% achieved the ability to walk without assistance. We expect 85% of treated patients ≥6 months and < 24 months of age to achieve the ability to stand alone and 60% to achieve the ability to walk without support. With this efficacy a sample size of 12 patients would provide power of > 90% to detect a significant difference compared with the matched control cohort with $\alpha = 0.05$ using a two-sample 2-sided superiority Fisher exact test.

The primary efficacy endpoint will be the change in HFMSE in treated patients between 24 months and < 60 months of age at time of dosing. Twelve children aged ≥ 24 months and < 60 months will be enrolled. Change in HFMSE score at 12 months will be compared with a cohort of eligibility-matched patients from the PNCR natural history study of SMA. Based upon a review of eligibility-matched patients from the PNCR, a mean change of -1.33 points (standard deviation = 4.32 points) is seen at 12 months from baseline among patients aged 2-5 years at first evaluation with 3 copies of *SMN2* in the PNCR dataset. We expect to observe a mean increase

of 8 points from baseline on the HFMSE with equivalent variance. Based upon these assumptions, 12 patients between 24 and <60 months at enrollment will have >90% power to detect a significant difference with $\alpha = 0.05$ when compared to patient-level data available from the PNCR dataset using two-sample ANOVA.

14.5. Demographic and Other Baseline Characteristics

Continuous demographic and other baseline characteristics (age, months since symptom onset, etc.) will be summarized by dosing cohort using n, mean, SD, median, minimum, and maximum. Qualitative characteristics (such as gender and race) will be summarized by counts and percentages.

14.6. Efficacy Analysis

14.6.1. Observation Period for Efficacy

Efficacy comparisons will be made once all patients have either completed 15 months of follow-up or withdrawn prior to 15 months of follow-up. The primary analysis of efficacy data will be based on data collected through the 12-month time point; a supportive analysis of efficacy data will be based on data collected through the 15-month time point.

14.6.2. Primary Efficacy Endpoints

Primary efficacy endpoints are assessed independently for each age group.

14.6.2.1. Primary Efficacy Endpoint: patients ≥6 months and < 24 months at time of dosing

The primary efficacy endpoint is the proportion of patients ≥6 months and < 24 months at time of dosing that achieve the ability to stand alone, without support for at least 3 seconds (Bayley Scales of Infant and Toddler Motor Development – Gross Motor subset item # 40) up to the 12-month study visit. A Responder for the primary endpoint of standing alone will be defined as per the Bayley Scales of Infant and Toddler Motor Development – Gross Motor subset item #40 – the child stands alone for at least 3 seconds after you release his or her hands.

The proportion of patients ≥6 months and < 24 months of age at time of dosing achieving the ability to stand without support up to the 12-month study visit will be compared between AVXS-101 and natural history control groups using a two-sample 2-sided superiority Fisher exact test. A 95% exact conditional confidence interval for the difference in response rates will be computed.

The primary analyses will be based on the Full Analysis Set for patients ≥6 months and < 24 months of age at the time of dosing.

A sensitivity analysis will be conducted by repeating each primary efficacy analysis on the Efficacy Completers Analysis Set for patients ≥ 6 months and < 24 months of age at the time of dosing.

A supportive analysis will be conducted by repeating the analysis of the primary efficacy endpoint for patients ≥ 6 months and < 24 months of age considering data through the 15-month visit.

14.6.2.2. Primary Efficacy Endpoint: patients ≥ 24 months and < 60 months at time of dosing

The primary endpoint is the change from baseline in HFMSE for patients ≥ 24 months and < 60 months of age at time dosing.

The primary efficacy analysis performed amongst patients aged between 24 months and < 60 months of age at time of dosing will be the change from baseline in HFMSE at the 12-month study visit using a two-sample ANOVA at significance level of 0.05 (2-sided). A 95% confidence interval will be computed.

The primary analyses will be based on the Full Analysis Set for patients ≥ 24 months of age and < 60 months of age at the time of dosing.

A sensitivity analysis will be conducted by repeating each primary efficacy analysis on the Efficacy Completers Analysis Set for patients ≥ 24 months of age and < 60 months of age at the time of dosing.

A supportive analysis will be conducted by repeating the analysis of the primary efficacy endpoint for patients ≥ 24 months of age and < 60 months of age considering data through the 15-month visit.

14.6.3. Secondary Efficacy Endpoint

The secondary endpoint for both patient strata (aged ≥6 months and < 24 months at dosing, aged ≥ 24 and < 60 months at dosing) will be the proportion achieving the ability to walk without assistance, defined as per the Bayley Scales of Infant and Toddler Motor Development – Gross Motor subset item # 43) up to the 12-month study visit. A Responder will be defined as a patient demonstrating achievement of the ability to walk without assistance at any post-procedure visit up to Month 12.

The proportion of patients ≥6 months and < 24 months or ≥ 24 and < 60 months at time of dosing who achieve the ability to walk without assistance will be compared separately between AVXS 101 and natural history control groups using two-sample Fisher's 2-sided exact test. A 95% exact confidence interval for the differences in response rates will be computed.

A supportive analysis will be conducted by repeating the analysis of the secondary efficacy endpoint considering data through the 15-month visit.

[redacted]

14.7. Safety Analysis

Safety will be assessed through monitoring AE reports and concomitant medication usage, and by conducting physical examinations, vital sign assessments, cardiovascular evaluations, and laboratory evaluations (chemistry, hematology, coagulation, immunology). Patients will be observed at the hospital for 48 hours post IT injection. Patients will return for follow-up visits in accordance with the SoA. Adverse events will be coded in accord with the most current version of the MedDRA coding dictionary.

14.7.1. Primary Safety Endpoints

14.7.1.1. Safety and Tolerability of AVXS-101 IT Administration

The safety and tolerability of IT administration of AVXS-101 will be assessed through the incidence, severity, and relatedness of AEs up to the time of data lock.

The proportion of patients with any AE, any SAE; any AE related to study product, and any Grade III or higher AE will be summarized overall, and by Body System and Preferred Term. Incidence rates will also be reported by cohort.

14.7.1.2. Determination of Optimal Dose

Up to 3 IT doses of AVXS-101 are examined in this dose escalation study. The DSMB/DMC's recommendation to escalate from Dose A to Dose B will be based on safety data as of the first quarterly DSMB/DMC meeting following enrollment of the third patient in Cohort 1. The recommendation to proceed to Cohort 2 signals the DSMB/DMC's evaluation that Dose A has acceptable safety.

Similarly, at the first quarterly DSMB/DMC meeting following enrollment of the third patient in Cohort 2, the DSMB/DMC will again evaluate safety data. The recommendation to expand Cohort 2 signals the DSMB/DMC's evaluation that the Dose B also has acceptable safety.

Based upon an ongoing assessment of safety and efficacy data from patients treated with Dose B, an option for testing of a third dose (Dose C), will be considered. If AveXis decides to proceed with testing of a third dose, a meeting of the DSMB/DMC will be convened to make recommendations regarding dose escalation. The DSMB/DMC's recommendation to escalate from Dose B to Dose C will be based on safety and efficacy. The recommendation to proceed to Cohort 3 signals the DSMB/DMC's evaluation that Dose B has acceptable safety.

14.7.2. Secondary Safety Endpoints

14.7.2.1. Ventilatory Support

The number of hours per day of non-invasive ventilatory support will be captured continuously by the device. The n, mean, median, range of number of hours per day of non-invasive ventilatory support will be summarized by visit and cohort. At each visit and within cohort, the daily non-invasive ventilatory support use will be summarized for AVXS- 101.

15. DATA SAFETY MONITORING BOARD/DATA MONITORING COMMITTEE

The DSMB/DMC is an independent multidisciplinary group consisting of clinicians and a biostatistician that, collectively, have experience in the management of patients with SMA Type 1 and other diseases, and in the conduct and monitoring of randomized clinical studies with interim analyses. The DSMB/DMC will be chartered to oversee the safety of patients during the conduct of the trial and will act in an advisory capacity to AveXis. A detailed description of the DSMB/DMC, its role in this trial, and the timing of the scheduled reviews will be described in a DSMB/DMC Charter.

The DSMB/DMC will routinely convene on a quarterly basis to review emerging safety data from the trial. All available safety data from all enrolled patients will be included in such reviews, which include, but are not limited to, screen failures, enrollment status, data from safety parameters, all SAEs, and other AEs. Following each meeting, the DSMB/DMC will make a recommendation as to whether or not the accumulated safety data warrants a suspension or discontinuation of the trial, a modification to the trial, or any additional comments or recommendations related to safety. The DSMB/DMC will prepare and provide minutes of their meetings to AveXis who will provide copies to the regulatory authorities as appropriate.

The DSMB/DMC will also convene on an ad hoc basis within approximately 48 hours should any patient experience an unanticipated CTCAE Grade 3 or higher AE/toxicity that is possibly, probably or definitely related to gene replacement therapy, and is associated with clinical symptoms and/or requires medical treatment. This includes any patient death, important clinical laboratory finding, or any complication attributed to administration of gene replacement therapy. In such instances, the DSMB/DMC will review the safety information and provide its recommendation. Based upon the DSMB/DMC's review, the DSMB/DMC can recommend continuing enrollment, halting enrollment or early termination of the trial for safety reasons.

15.1. DSMB/DMC Interim Safety Review

Up to three interim safety reviews will be conducted by the DSMB/DMC. The first DSMB/DMC interim safety review will take place after three patients have been enrolled in Cohort 1. The consideration for this review is terminating the study for safety. In the absence of a termination recommendation, a recommendation to escalate enrollment to the higher dose is made.

The second DSMB/DMC interim safety review will take place after three patients have been enrolled in Cohort 2 (six total enrolled). The consideration for this review is terminating enrollment in Cohort 2 due to safety concerns. In the absence of a termination recommendation, a recommendation to expand the current Cohort 2 is made.

The third DSMB/DMC interim safety review may take place after three patients have been enrolled in Cohort 3. The consideration for this review is terminating enrollment in Cohort 3 due to safety concerns. In the absence of a termination recommendation, a recommendation to expand the current Cohort 3 is made.

16. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

16.1. Study Monitoring

Before an investigational site can enter a patient into the study, a representative of AveXis will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of AveXis or its representatives. This will be documented in a Clinical Study Agreement between AveXis and the Investigator.

During the study, a monitor from AveXis or representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the Investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the eCRFs, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the eCRFs with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (e.g., clinic charts)
- Record and report any protocol deviations not previously sent to AveXis
- Confirm AEs and SAEs have been properly documented on eCRFs and confirm any SAEs have been forwarded to AveXis, Inc. and those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the Investigator(s) or other staff need information or advice.

16.2. Audits and Inspections

Authorized representatives of AveXis regulatory authorities, an IRB/IEC may visit the site to perform audits or inspections, including source data verification. The purpose of an AveXis audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines of the International Council for Harmonisation (ICH), and any applicable regulatory requirements. The Investigator should contact AveXis immediately if contacted by a regulatory agency about an inspection.

16.3. Institutional Biosafety Committee

As this study involves gene therapy, the Principal Investigator must obtain approval/favorable opinion for the investigation from a designated institutional or independent biosafety committee in accord with institutional requirements and/or guidelines.

16.4. Institutional Review Board/Independent Ethics Committee

The Principal Investigator must obtain IRB/IEC approval for the investigation. Initial IRB/IEC approval, and all materials approved by the IRB/IEC for this study including the patient / subject consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

17. QUALITY CONTROL AND QUALITY ASSURANCE

Qualified individuals designated by AveXis will monitor all aspects of the study according to GCP, standard operating procedures (SOPs), and for compliance with applicable government regulations. Please see Section 16.1 for more details regarding the quality control and monitoring process. AveXis may also conduct a quality assurance audit any time during or after the completion of the study. Please see Section 16.2 for more details regarding the audit process.

The Investigator agrees to allow these AveXis representatives direct access to the clinical data and supplies, dispensing and storage areas and if requested, agrees to cooperate fully or assist the AveXis representative. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by AveXis or its designees.

Noncompliance with the protocol, ICH, GCP or local regulatory requirements by an Investigator, site staff, or representatives of AveXis will lead to prompt action by the AveXis to secure compliance. Continued noncompliance may result in termination of the corresponding party's involvement in the study. The IRB/IEC and relevant regulatory authority will also be informed.

18. ETHICS

18.1. Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB/IEC as appropriate. The Investigator must submit written approval to AveXis before he/she can enroll any patient into the study.

The Principal Investigator is responsible for informing the IRB/IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB/IEC must approve all advertising used to recruit patients for the study. The protocol must be re-approved by the IRB/IEC upon receipt of amendments and annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. AveXis or designee will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB/IEC according to local regulations and guidelines.

Initial IRB approval, and all materials approved by the IRB/IEC for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

18.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (please see Appendix 1) and are consistent with ICH GCP, and applicable regulatory requirements.

18.3. Written Informed Consent

The Principal Investigator(s) at each center will ensure that the patient/parent(s)/legal guardian(s) is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients/parent(s)/legal guardian(s) must also be notified that they are free to discontinue from the study at any time. The patient/parent(s)/legal guardian(s) should be given the opportunity to ask questions and allowed time to consider the information provided.

The patients' signed and dated informed consent must be obtained before conducting any study procedures.

The Principal Investigator(s) must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the patient.

There will be three Informed Consent Forms:

- Parent/Guardian Informed Consent Form
- Biological Mother Baseline AAV9 Antibody Screening Informed Consent Form
- Autopsy Informed Consent Form (optional)

19. DATA HANDLING AND RECORDKEEPING

19.1. Electronic Case Report Forms

Adequate and accurate case records will be maintained, and all relevant observations and data related to the study will be recorded. This will include medical history/ physical examination, hematology, clinical chemistry and serology results, a check list of inclusion and exclusion criteria, urinary drugs of abuse screening, product administration, and a record of sample collection, hemodynamic measurements, clinical assessments, AEs, and final evaluation.

Electronic CRFs will be used in this study. The eCRF will be electronically signed and dated by the Principal Investigator, or his designee, after his/her review. After the completion of the study, completed eCRFs will be retained in the archives.

Completed eCRFs will be reviewed by the study monitor against the source documentation for accuracy and completeness. Once signed by the Investigator, the monitor will transmit the completed eCRFs to data management for data validation and database analysis.

19.2. Inspection of Records

AveXis or designee will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the product storage area, study product stocks, product accountability records, patient charts and study source documents, and other records relative to study conduct.

19.3. Retention of Records

All primary data that are a result of the original observations and activities of the study and that are necessary for the reconstruction and evaluation of any study report will be retained in a secure archive at the study site for a period not less than 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have lapsed since the formal discontinuation of the clinical development of the investigational product. All country/region specific requirements that may be more stringent than the 2 years (e.g., 25 years in Canada) included in ICH shall be followed.

The site will maintain essential documents as required by ICH-GCP. The site must keep these documents available for review by AveXis, IRB/IEC, and/or regulatory bodies.

20. PUBLICATION POLICY

The Investigator is obliged to provide AveXis with complete test results and all data derived by the Investigator from the study. During the study, only AveXis may make study information available to other study Investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the clinical study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of AveXis.

AveXis may publish any data and information from the study (including data and information generated by the Investigator) without the consent of the Investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the Clinical Study Site Agreement. In the event of any discrepancy between the protocol and the Clinical Study Site Agreement, the Clinical Study Site Agreement will prevail.

If the study is being conducted as part of a multicenter clinical study, data from all sites participating in the study will be pooled and analyzed by AveXis or the AveXis designee. The first publication of the study results shall be made in conjunction with the results from other study sites as a multicenter publication. If a multicenter publication is not forthcoming within 24 months of completion of the study at all sites, the Investigator may publish or present the results generated at his/her site.

The Investigator will provide the AveXis with a copy of any proposed publication or presentation for review and comment at least 60 days prior to such presentation or submission for publication. AveXis shall inform the Investigator in writing of any changes or deletions in such presentation or publication required to protect AveXis confidential and proprietary technical information and to address inaccurate data or inappropriate interpretations in the context of any pooled multicenter results. At the expiration of such 60-day period, the Investigator may proceed with the presentation or submission for publication unless AveXis has notified the institution or the Investigator in writing that such proposed publication or presentation discloses AveXis confidential and proprietary technical information. Further, upon the request of AveXis, the Investigator will delay the publication or presentation for an additional 90 days to permit AveXis to take necessary actions to protect its intellectual property interests.

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22. APPENDICES

APPENDIX 1. DECLARATION OF HELSINKI ETHICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.
10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.
11. Medical research should be conducted in a manner that minimizes possible harm to the environment.
12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.
17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

    Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.
18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

    When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

    All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

    The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

    In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards, but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

    The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any SAEs. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

    After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

    All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics

committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorized representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

    Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention. Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist, or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

APPENDIX 2. HAMMERSMITH FUNCTIONAL MOTOR SCALE-EXPANDED

Smartnet & PNCR	Hammersmith Functional Motor Scale for SMA (HFMS)						07/03/09
Name			DOB				
Date of assessment			Time taken to complete				
Date of spinal surgery			Evaluator				
Please circle highest current level of independent mobility None Rolls Bottom shuffles creeps /crawls Walks with crutches / frame /rollator Walks with KAFO's / AFO's Independent walking Comment.....					LBC = Limited by contracture		
Test	***Instruction***	**2**	**1**	**0**	**L B C**	**S =**	**Comments** **S = score**
1 Plinth /chair sitting Can be over edge of plinth or on plinth / floor. Record best you see	*Can you sit on the plinth /chair without using your hands for support for a count of 3?(Back unsupported /feet +/- support)*	Able to sit using no hand support for a count of 3 or more	Needs one hand support to maintain balance for a count of 3	Needs two hand support to maintain balance Unable to sit			**Item 1** Predominant spinal posture Predominant leg posture Circle predominant spinal posture and leg position
2 Long sitting Legs straight = knees maybe flexed, knee caps pointing upwards, ankles <10cm apart	*Can you sit on the floor/plinth without using your hands for support and with your legs straight for a count of 3?*	Able to sit on floor/plinth with legs straight without hand support for a count of 3	Able to sit on floor/plinth with legs straight propping with one hand support for a count of 3	Able to long sit using two hands for a count of 3 Or unable to sit with straight legs			
3 One hand to head in sitting Hand touch head above level of ears	*Can you get one hand to your head without bending your neck*	Able to bring one hand to head. Head and trunk remain stable	Can only bring hand to head by flexing head	Unable to bring hand to head even using head and trunk movement			R / L
4 Two hands to head in sitting Hands touch head above level of ear	*Can you lift both hands up at the same time, to your head, without bending your neck?*	Able to place both hands on head arms free from side. Head and trunk remain stable	Able to place hands on head but only using head flexion or side tilt or crawling hands up or one at a time	Unable to place both hands on head			
5 Supine to side-lying	*Can you roll onto your side in both directions? Try not to use your hands*	Able to ½ roll from supine both ways	Can ½ roll only one way R / L	Unable to half roll either way			Shoulders perpendicular to floor. Trunk and hips in line with body
6 Rolls prone to supine over R	*Can you roll from your tummy to your back in both directions?*	Turns to supine with free arms to the right	Turns to supine using arms to push/ pull with	Unable to turn into supine			
7 Rolls prone to supine over L		Turns into supine with free arms to the left	Turns to supine using arms to push/ pull with	Unable to turn into supine			
8 Rolls supine to prone over R	*Can you roll from your back to your front in both directions?*	Turns to prone with free arms to the right	Turns to prone by pulling/ pushing on arms	Unable to turn into prone			
9 Rolls supine to prone over L		Turns to prone with free arms to the left	Turns to prone by pulling/ pushing on arms	Unable to turn into prone			
10 Sitting to lying	*Can you lie down in a controlled way from sitting?*	Able to lie down in a controlled fashion through side lying or using clothes	Able to lie down by flopping forwards and rolling sideways	Unable or falls over			
11 Props on forearms	*Can you prop yourself on your forearms and hold for a count of 3?*	Able to achieve prop on elbows with head up for a count of 3	Holds position when placed for a count of 3	Unable			
12 Lifts head from prone	*Can you lift you head up keeping your arms by your side for a count of 3?*	Able to lift head up in prone arms by side for a count of 3	Lift head with arms in a forward position for a count of 3	Unable			
13 Prop on extended arms	*Can you prop yourself up with straight arms for a count of 3?*	Able to prop on extended arms, head up for a count of 3	Can prop on extended arms if placed for a count of 3	Unable			
14 Lying to sitting	*Can you get from lying to sitting without rolling to your tummy?*	Able by using side lying	Turns into prone or towards floor	Unable			
15 Four-point kneeling	*Can you get onto your hands and knees with your head up and hold for a count of 3?*	Achieves four-point kneeling – head up for a count of 3	Holds position when placed for a count of 3	Unable			

Test	*Instruction*	2	1	0	L B C	S =	Comments S = score
16 Crawling	*Can you crawl forwards?*	Able to crawl forwards – moves all four points twice or more	Moves all four points only once	Unable			
17 Lifts head from supine	*Can you lift your head to look at your toes keeping your arms folded for a count of 3*	In supine, head must be lifted in mid-line. Chin moves towards chest. Held for a count of 3	Head is lifted but through side flexion or with no neck flexion Held for a count of 3	Unable			
18 Supported standing	*Can you stand using one hand for support for a count of 3?*	Can stand using one hand support for a count of 3	Able to stand with minimal trunk support (not hip) for a count of 3	Can stand with hand support but needs knee/hip support in addition for a count of 3 Or unable			
19 Stand unsupported	*Can you stand without holding onto anything for a count of 3?*	Can stand independently for the more than a count of 3	Stands independently for a count of 3	Stands only momentarily (less than a count of 3) Or unable			
20 Stepping	*Can you walk without using any help or aids? Show me*	Able to take more than 4 steps unaided	Able to take 2 – 4 steps unaided	Unable			
SCORE		**No of 2's =**	**No of 1's =**	**No of 0's =**	**TOTAL =**		**/40**
Comments							

PNCR	Expanded Hammersmith Functional Motor Scale for SMA (HFMSE) *add-on module*						07/03/09
Test	***Instruction***	**2**	**1**	**0**	**L B C**	**S =**	**Comments S = score**
21 Right hip flexion in supine	*Can you bring your right knee to your chest?*	Full hip flexion achieved	Initiates right hip and knee flexion (more than 10% of available range of motion)	Unable			
22 Left hip flexion in supine	*Can you bring your left knee to your chest?*	Full hip flexion achieved	Initiates left hip and knee flexion (more than 10% of available range of motion)	Unable			
23 High kneeling to right half kneel	*Can you bring your **left** leg up so that your foot is flat on the ground without using your arms and hold for a count of 10?*	Arms used for transition, maintains arms free in half kneel for a count of 10	Maintains half kneel with arm support for a count of 10	Unable			
24 High kneeling to left half kneel	*Can you bring your **right** leg up so that your foot is flat on the ground without using your arms and hold for a count of 10?*	Arms used for transition, maintains arms free in half kneel for a count of 10	Maintains half kneel with arm support for a count of 10	Unable			
25 High kneeling to stand leading with left leg	*Can you stand up from this position starting with your left leg without using your hands? May need demonstration*	Able with arms free	Able to shift weight off both knees (with or without arm support)	Unable			
26 High keeling to stand leading with right leg	*Can you stand up from this position starting with your right leg without using your hands? May need demonstration*	Able with arms free	Able to shift weight off both knees (with or without arm support)	Unable			
27Stand to sit	*Can you sit on the floor, in a controlled way? Try not to use your arms.*	Able to sit down with arms free and no collapse	Sits on floor but uses arms or crashes	Unable			
28Squat	*Can you squat? Pretend you are going to sit in a very low seat.*	Squats with arms free (at least 90° of hip and knee flexion)	Initiates squat (more than 10%) , uses arm support	Unable to initiate			
29 Jump 12" forward	*Can you jump as far as you can, with both feet, from this line all of the way to the other line?*	Jumps at least 12", both feet simultaneously	Jumps between 2-11", both feet simultaneously	Unable to initiate jump with both feet simultaneously			
30 Ascends stairs with rail	*Can you walk up the steps? You can use one railing*	Ascends 4 stairs with railing, alternating feet	Ascends 2-4 stairs, one rail, any pattern	Unable to ascend 2 stairs one rail			
31 Descends stairs with rail	*Can you walk down the steps? You can use one railing*	Descends four stairs , with railing, alternating feet	Descends 2-4 stairs, one rail, any pattern	Unable to descend 2 stairs with one rail			
32 Ascends stairs without rail	*Can you walk up the steps? This time try not to use the railing*	Ascends four stairs, arms free, alternating feet	Ascends 2-4 stairs, arms free, any pattern	Unable to ascend 2 stairs arms free			
33 Descends stairs without rail	*Can you walk down the steps? This time try not to use the railing*	Descends four stairs, arms free, alternating feet	Descends 2-4 stairs, arms free, any pattern	Unable to descend 2 stairs arms free			
SCORE		**No of 2's =**	**No of 1's =**	**No of 0's =**			**TOTAL = /66**

APPENDIX 3. SUMMARY OF CHANGES

The section below highlights content changes represented in this version of the protocol. Language deleted from Protocol version 7.0 appears in ~~red strike through~~. Language added to Protocol version 8.0 appears in **bold**.

Administrative changes have been made throughout the entire protocol, including updated references and minor editorial edits (minor changes in formatting, spelling, punctuation, grammar, order of first appearance of abbreviations, etc.). Time points for procedures within text of the protocol were deleted and replaced with a statement referencing Schedule of Assessments.

The Amendment 7 version of the protocol (Protocol version 8.0) is updated to include more stringent eligibility criteria for liver function tests and additional visits for monitoring liver function, following the acute liver failure case reported in the US Managed Assess Program.

General

References updated throughout the protocol.

General

Minor editorial edits throughout the protocol.

Rationale for change

Updated references as part of administrative changes.

Section 1.1. Approval

This study will be conducted with the highest respect for the individual patients in accordance with the requirements of this clinical study protocol and in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki
- International Council for Harmonisation ~~and Harmonised Tripartite~~**, Integrated Addendum to ICH E6(R1):** Guideline for Good Clinical Practice **E6 (R2)**
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations

SIGNATURES: (may be applied electronically and will therefore be maintained in the electronic system):

[redacted] _____ _____
Date
Vice President of Clinical Development
AveXis, Inc.

[redacted] _____ _____
Date

Sr. Medical Director, Clinical Development
AveXis, Inc.

[redacted] Date
~~Interim Head~~ **Senior Director** of Clinical Operations
AveXis, Inc.

[redacted] Date
Head of Biostatistics
AveXis, Inc.

[redacted]
VP & Head, Global Patient Safety
AveXis, Inc.

Rationale for change:

Updated signatures of representatives from Avexis as part of administrative changes.

Section 1.4 Key Contact Information

Responsible Physician	[redacted] **Medical Director, Clinical Development,**
Serious Adverse Event Reporting	[redacted]

Additional study contact information is provided in the Study Contact List.

~~Table 1: Important Study Contact Information~~

~~Role in Study~~	~~Name/ Address and Telephone Number~~
~~Clinical Study Leaders~~	~~Please see INC Project Communication Plan in Trial Master File (TMF) or Study Contact list in the Investigator Site File (ISF)~~
~~Responsible Physician~~	~~Please see INC Project Communication Plan in Trial Master File (TMF) or Study Contact list in the Investigator Site File (ISF)~~
~~Drug Safety Physician 24 Hour Emergency Contact~~	~~Please see INC Project Communication Plan in Trial Master File (TMF) or Study Contact list in the Investigator Site File (ISF)~~
~~SAE Reporting~~	~~INC~~

~~Table 2: Study Vendor Listing~~

~~Role in Study~~	~~Name/Address~~
~~Clinical Research Organization~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~
~~Central Laboratories~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~
~~Video~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~
~~IP Shipment~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~
~~Non-invasive ventilatory support device~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~
~~Independent Video Review~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~
~~Holter Monitor~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~
~~Autopsy~~	~~Please see INC Project Management Plan in TMF or Study Contact list in ISF~~

Rationale for change

Updated key contact information and removed other specific contact information as part of administrative changes.

Section 2 SYNOPSIS

Section 2 was updated as per changes throughout the document.

Section 5.2. Rationale for Gene Transfer to SMA Type 2/3 Patients

Table 2 Footnote

Source: Adapted from Kolb 2011.
SMN2 = survival motor neuron 2 gene
bold = predominant SMN2 copy number that defines the SMA Type, the other copy numbers represent a small percentage of the designated SMA Type.

Rationale for change

Updated to include footnote specifying the most frequent presentation of the SMA copy number.

Section 5.3 Non-Clinical Studies

Activity of the scAAV9.CB.SMN was demonstrated by the bio distribution and the presence of a specific transgene ribonucleic acid (RNA) expression in brain and spinal cord, the main targeted therapeutic tissues. Low levels of antibodies to the AAV9 capsid were found after 12 and 24 weeks in males and females given 3.3 × 1014 vg/kg (Group 3). No alteration was observed in clinical pathology and histopathology analyses. ~~Based on these results, the no observable adverse effect level (NOAEL) of scAAV9.CB.SMN in newborn male and female mice is considered to be 3.3 × 1014 vg/kg.~~

Rationale for change

Updated to remove redundancy within the protocol.

Section 5.4 Clinical Studies

First-in-human trial AVXS-101-CL-101 is a completed 2-year trial which evaluated the efficacy and safety of AVXS-101 in 15 SMA Type 1 patients with 2 copies of *SMN2*. All patients received a single IV dose of AVXS-101 in 2 cohorts (Mendell, 2017). After the End of Trial visit, patients were invited to participate in a long term follow up study conducted under a separate protocol.

Based on data obtained in Study AVXS 101-CL-101, the following conclusions can be made regarding the efficacy of AVXS-101: AVXS-101 administration had a positive effect on survival. Twenty-four months after dosing, all 15 patients were alive and free of permanent ventilation and all Cohort 2 patients had survived free of permanent ventilation, a statistically significant difference compared with the natural history rate of 8% reported by Finkel, 2014 (Finkel et al 2014).

Rationale for change

Added information based on current studies

Section 5.5 Risks

A full understanding of all risks of AVXS-101 is not known at this time. Potential risks of AVXS-101 are discussed below and further details are provided in the Investigator's Brochure. Please note, the risks discussed below are the result of observations with IV administration. The dose for IV therapy is 1- to 3-fold higher than for the IT therapy. Because the IT dose is lower than the IV dose and delivered directly to the CNS, the systemic exposure and associated risks are expected to be less.

Patients could experience an allergic response to AVXS-101. Patients could also develop an immune response to the AAV9 viral vector, which could prevent future use of gene transfers using this 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 SMA is unknown.

Respiratory tract infections in neonates are very common in the general pediatric population (Paes, 2001). In one study, an estimated 338 million new episodes of RSV-associated acute lower respiratory infections occurred worldwide in children younger than 5 years, with at least 34 million episodes necessitating hospital admission (Nair, 2010). Respiratory syncytial virus is the primary cause of hospitalization for respiratory tract infection in young children (Hall, 2001). It would not be unexpected that infants enrolled

in the AVXS-101 gene replacement therapy trials might have similar incidences of respiratory infections due to these pathogens.

Patients must be clinically stable before AVXS-101 dosing. Clinical signs or symptoms of infection should not be evident at the time of AVXS-101 administration. Vaccinations, including palivizumab prophylaxis that can prevent RSV infections (Palivizumab SmPC, April 2019), are also recommended (Finkel et al 2017) and should be up to date. Added caution is advised regarding the timing of AVXS-101 administration in the presence of prodrome or resolving viral illness. In the event of a severe viral respiratory infection, the Investigator should be aware of the possibility of adrenal insufficiency in the presence of systemic immune response which may require longer glucocorticosteroid support at increasing doses to effectively manage the patient and prevent serious complications.

Some mice affected with SMA Type 1 that were treated with AVXS-101 experienced liver findings comprised on hepatocellular hypertrophy, Kupffer cell activation, and scattered hepatocellular necrosis.

Adverse events (AEs) of increased transaminases (ALT increased, AST increased, and transaminase increased) were reported as related to AVXS-101 in clinical studies. The underlying cause of the transaminase elevations is not known; though, may be related to an immune response to AAV9, as indicated by the response to prednisolone. Though these AEs can be serious, in most cases they were clinically asymptomatic, did not meet criteria for Hy's law, did not exhibit clinically relevant increases in bilirubin, and generally resolved with prednisolone treatment. A case of acute liver failure (serious unexpected serious adverse reaction [SUSAR]) was reported in the US Managed Access Program with AVXS-101. A 6-month-old male concurrently receiving nusinersen with elevations of AST and ALT of > 3 x upper limit of normal (ULN) before treatment with AVXS-101 developed acute liver failure approximately 51 days post AVXS-101 dosing. The patient recovered with additional steroid therapy.

Nonclinical cardiovascular toxicity findings that could potentially be relevant to the clinical use of AVXS-101 have been reported in 2 mouse toxicology studies of AVXS-101. Similar findings were reported in both studies. Findings in the ventricles of the heart were comprised of inflammation, edema and fibrosis. Primary findings in the atrium of the heart were thrombosis and inflammation. The underlying mechanism of these findings and the translatability of the observed findings in mice to primates are not known at this time.

The available clinical cardiovascular safety data have not provided evidence for a cardiovascular safety problem in humans. As of the last update to the Investigator's Brochure (v5), there have been no cardiovascular AEs that have been judged to be related to AVXS-101 in the clinical studies.

A transient decrease in platelet counts has been observed in both IV and less frequently with IT administration. The majority of values remained above the lower limit of normal. Decreases were clinically asymptomatic and transient.

This risk can be effectively managed through monitoring platelet counts for 1 month following dosing, and appropriate prednisolone (or an equivalent corticosteroid in countries where prednisolone is not available) use.

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 wtAAV, 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 wtDNA removed from the capsids, except for the inverted terminal repeats (ITRs), 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 wtAAV2 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. For a review of the topic, see Srivastava and Carter, 2017 (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 wtAAV2. However, the rescue, replication, and packaging would stop 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 recombinant AAV (rAAV) genome can be rescued and replicated by superinfection with wwtAAV 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.

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 caregivers 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.

Viral shedding is dependent on a variety of factors including the route of administration of the product, the tropism of the virus or bacteria, and the natural route of transmission and shedding of the parent virus or bacterium from which the product is derived. AveXis collected saliva, urine, and stool samples at weekly timepoints through Day 30 and then monthly timepoints through Month 18 after gene transfer during the AVXS-101-CL-101 clinical study from five patients for viral shedding analysis. This analysis detects the number of genome copies by droplet digital polymerase chain reaction (ddPCR) in the applicable shed samples.

After IV administration, AVXS-101 is detectable in the shed samples from Day 1 post injection. All five patients analyzed were dosed with 2.0×10^{14} vg/kg. Concentrations of vector shed in saliva and urine are quite low and are below the limits of quantitation by ddPCR in the matrices within days post dose. Shedding of AVXS-101 was reported as a proportion of the initial concentration. All five patients analyzed for viral shedding were dosed with the proposed therapeutic dose. AVXS 101 was detectable in shed samples post-infusion. 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, three patients for whom data were available showed a concentration of <1% of initial concentration in body at day 14 post infusion, with concentrations declining approximately 4 logs (10,000-fold) over 30 days post infusion. Overall, AVXS-101 was primarily cleared from the body in stool and by day 60 post infusion was below the limit of quantitation in stool. Shed AAV vectors have been previously shown not to be infectious in urine and saliva excreta (Favre, 2001). Together, these data demonstrate rapid decline of shed vector quantities well below initial concentrations in patients treated with AVXS-101. Clearance of AVXS-101 is primarily via the feces and the majority of the dose is cleared within 30 days of dose administration.

The results seen in the IV development program support further clinical investigation of the efficacy and safety of AVXS-101 in patients with SMA. Based on the current available data, safety events that appear to be associated with AVXS-101 consist of transient liver enzyme elevations, which have resolved following treatment with prednisolone.

Taken together, results from the clinical and non-clinical studies support further clinical investigation of the efficacy and safety of AVXS-101 in patients with SMA.

Rationale for change

Added section to include description of recent adverse events and to include benefit/risk language from the IB and the Clinical Overview and also to include information on the acute liver failure case reported in the US Managed Access Program.

Section 7.1 Overall Study Design

The first cohort will enroll three ~~(3)~~ patients (Cohort 1) ≥6 months and < 24 months of age who will receive administration of 6.0×10^{13} vg of AVXS-101 (Dose A). There will be at least a four

~~(4)~~ week interval between the dosing of each patient within the cohort. ~~The investigators~~**AveXis** will confer with the Data Safety Monitoring Board (DSMB) on all Grade III or higher AEs within **approximately** 48 hours of awareness that are possibly, probably, or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB during quarterly meetings; following enrollment of the first three patients and based upon the available safety data a decision will be made whether to: a) stop due to toxicity, or b) proceed to Cohort 2 using Dose B.

Should the determination be made to advance to Dose B, three ~~(3)~~ patients < 60 months of age will be enrolled to receive administration of 1.2×10^{14} vg of AVXS-101 (Dose B). Again, there will be at least a 4-week interval between dosing of the three patients within the cohort. Based on the available safety data from the three Cohort 2 patients and all of the Cohort 1 patients, the DSMB may decide and document during quarterly meetings that further 4-week intervals between patients dosing is unnecessary. AveXis~~, Inc.~~ will take this recommendation into consideration and will make the final determination whether to persist with 4-week intervals between patients dosing going forward; the decision will be communicated to sites and Institutional Review Board (IRBs) in a formal sponsor letter. ~~The investigators~~**AveXis** will confer with the DSMB on all Grade III or higher AEs within **approximately** 48 hours that are possibly, probably, or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB during quarterly meetings; following enrollment of the first six (6) patients and based upon available safety data, a decision will be made whether to a) stop due to toxicity, or b) continue to enroll an additional 21 patients until twelve (12) patients ≥6 months and < 24 months and ~~twelve (~~12~~)~~ patients ≥ 24 months and < 60 months have received Dose B.

Based upon an ongoing assessment of safety and efficacy data from patients treated with the 1.2×10^{14} vg dose, an option for testing of a third dose (Dose C), will be considered. If, based on all available data, this is judged to be necessary, three ~~(3)~~ patients < 60 months of age will receive Dose C, ~~which will be no greater than~~ 2.4×10^{14} vg administered IT. A meeting of the DSMB will be called to obtain agreement on the safety of escalating to a higher dose prior to proceeding. If agreement is obtained from the DSMB, there will again be a four-week interval between dosing of the first three patients receiving Dose C, as in Cohorts 1 and 2. Safety data will be reviewed by the DSMB during quarterly meetings; following enrollment of the first three ~~(3)~~ Dose C patients and based upon available safety data, a decision will be made whether to: a) stop due to toxicity, or b) continue to enroll an additional 21 patients until there are a total of twelve (12) patients > 6 months and < 24 months and twelve (12) patients ≥ 24 and < 60 months that have received Dose C.

Selection of the appropriate dose and justification for testing Dose C will be supported by ongoing safety and efficacy reviews of clinical findings from the patients receiving Dose B (1.2×10^{14} vg). ~~The selected dose will be no greater than~~**Dose C will be** 2.4×10^{14} vg delivered IT. Doses up to 1.1×10^{14} vg/kg have been safely administered systemically (IV) to children weighing up to 8.4 kg (total dose 9.24×10^{14} vg). In addition, in preclinical studies, the IT administration of scAAV9.CB.SMN was safe and well tolerated up to 14 months post injection in large non-human primates at a dose of 2×10^{13} vg/kg (~~AveXis~~ AVXS-101 Investigator's Brochure~~, Edition 4, page 19~~).

The overall study design is summarized in Figure 4.

Safety will be assessed through monitoring AE reports and concomitant medication usage, and by conducting physical examinations, vital sign assessments, cardiovascular evaluations, and laboratory evaluations. Patients will be observed at the hospital for 48 hours post IT injection. Patients will return for follow up ~~visits on Days 7, 14, 21, and 30. Patients will return monthly thereafter, following the Day 30 visit, for 12 months from dose administration~~**according to the Schedule of Assessments (SoA) (Table 3).**

Patients enrolled in Cohort 1 (Dose A) and Cohort 2 (Dose B) will complete 12 months of follow-up post IT administration. Patients enrolled in Cohort 3 (Dose C) will complete 15 months of follow-up post IT administration.

An autopsy and tissue collection process will be in place for those patients who consent to autopsy/tissue collection for research purposes.

Once patient completes the study, he or she will be invited to participate in a long-term follow-up study ~~examining the lasting safety of AVXS-101 up to 15 years~~ conducted under a separate protocol.

Table 8: Schedule of Assessments

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
~~Visit #~~	~~1~~	~~2~~			~~3~~	~~4~~	~~5~~	~~6~~				~~Monthly (7-16)~~	~~Month 12/ EOS (17)~~	
~~# of Days/Months in~~ Study Day	-60 to -2	-1	1	2-3[1]	7	14	21	30	~~374~~ 4	60	~~517~~ 2	Monthly Through ~~Month~~M ~~1~~14	M~~onth~~1 ~~2~~5/EOS	
Window					~~+/-~~ 2							~~+/-~~ 7		
Informed Consent	X													
Spinal X-ray	X													
Demographics/ Medical History[12]	X		X	(X)	X	X	X	X		X		X	X	Medical history collected at baseline; review of systems conducted at each study visit. (X): Patients to remain inpatient 48 hours post-dose for observation and AE monitoring.
Physical Exam[20]	(X)[3]		X	X	X	X	X	X		X		X	X	Includes measuring head circumference. (X): Baseline procedure must be completed within 30 days of dosing.
Vitals/Weight/ Length/Height[4]	(X)[3]		X	X	X	X	X	X		X		X	X	Vital signs will include BP, respiratory rate, pulse, and axillary temperature. Vitals including BP, respiratory rate, pulse axillary temperature, pulse oximetry and HR will be monitored and recorded every 15 minutes (± 5 minutes) from dosing for four hours and every hour (± 15 minutes) for 24 hours following the AVXS-101 dosing procedure. (X): Baseline procedure must be completed within 30 days of dosing.
Pulse Oximetry	(X)[3]		X	X	X	X	X	X		X		X	X	(X): Baseline procedure must be completed within 30 days of dosing.
Pulmonary Exam	(X)[3]							X		X		X	X	

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
~~Visit #~~	~~1~~	~~2~~			~~3~~	~~4~~	~~5~~	~~6~~				~~Monthly (7-16)~~	~~Month 12/ EOS (17)~~	
~~# of Days/Months in~~ Study Day	-60 to -2	-1	1	2-3[1]	7	14	21	30	~~374~~ 4	60	~~517~~ 2	Monthly Through ~~Month~~M 1~~14~~	M~~onth~~1 ~~25~~/EOS	
Window					~~+/-~~ 2							~~+/-~~ 7		
12-Lead ECG[18]	X		X	X								(X)[2]	X	Irrespective of study schedule, after signing the updated informed consent, all patients enrolled prior to the Amendment 5 version of the protocol should have ECG, 24-hour Holter monitoring, and echocardiogram done at the next scheduled visit and then in conformity with the study ~~schedule of assessments~~SoA. (X): Months 3, 6, and 9 visits.
12-Lead Holter Monitor[5]	X	X	X	X				X		(X)		(X)[17]	X	ECGs extracted from Holter by central reader in triplicate at the following time points: pre-dose, 2h, 4h, 6h, 8h, 12h, 24h, 36h, and 48h during the patient's inpatient stay. (X): Months 2, 3, 6, 9 visits.
Echocardiogram[18]	X											(X)[2]	X	Irrespective of study schedule, after signing the updated informed consent, all patients enrolled prior to the Amendment 5 version of the protocol should have echocardiogram done at the next scheduled visit and then in conformity with the study ~~schedule of assessments~~SoA. (X): Months 3, 6, and 9 visits.
Capillary Blood Gas		X		(X)[15]										(X): Laboratory samples collected on Day 2.

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
~~Visit #~~	~~1~~	~~2~~			~~3~~	~~4~~	~~5~~	~~6~~				~~Monthly (7-16)~~	~~Month 12/ EOS (17)~~	
~~# of Days/Months in~~ Study Day	-60 to -2	-1	1	2-3~~1~~	7	14	21	30	~~374~~ 4	60	~~517~~ 2	Monthly Through ~~Month~~M 1~~14~~	M~~onth~~1 ~~25~~/EOS	
Window					~~+/-~~ 2							~~+/-~~ 7		
HFMS- Expanded~~10~~ (with video)	(X)~~3~~							X				X	X	Will be conducted for patients ≥ 24 months of age; patients < 24 months of age at study entry will begin HFMS-E at such time that 24 months of age is reached. (X): Baseline procedure must be completed within 30 days of dosing.
Bayley III (with video)[6]	(X)~~3~~							X		X		X[6]	X	Gross and fine motor sections will be performed at screening and monthly through 15 months. (X): Baseline procedure must be completed within 30 days of dosing.
Motor Milestone Development Survey (with video)	(X)~~3~~							X		X		X	X	
Hematology / Chemistry	(X)~~3~~	(X)*~~11~~		(X)~~$15~~	X	X	X	X	(X)†	X	(X)†	X	X	(X): Baseline procedure must be completed within 30 days of dosing. (X)*: Laboratory samples collected prior to dosing are to be processed locally. (X)~~$~~: Laboratory samples collected on Day 2. (X)† Liver function test (AST, ALT, total bilirubin, alkaline phosphatase, GGT) only.
Coagulation	(X)~~3~~	(X)*~~11~~		(X)~~$15~~	X	X	X	X		X		X	X	
Urinalysis	(X)~~3~~	(X)*~~11~~		(X)~~$15~~	X	X	X	X		X		X	X	

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
~~Visit #~~	~~1~~	~~2~~			~~3~~	~~4~~	~~5~~	~~6~~				~~Monthly (7-16)~~	~~Month 12/ EOS (17)~~	
~~# of Days/Months in~~ Study Day	-60 to -2	-1	1	2-3[1]	7	14	21	30	~~374~~ 4	60	~~517~~ 2	Monthly Through ~~Month~~M 1~~14~~	M~~onth~~1 ~~25~~/EOS	
Window					~~+/-~~ 2							~~+/-~~ 7		
CK-MB[19]	X				X			X		X		(X)[16]	X	Troponin I will be measured instead of CK-MB in new patients who are screened and enrolled after amendment 5 (protocol version 6.0) goes into effect. Participants who have been screened and enrolled but who have not yet received gene replacement therapy (visit #2) at the time that amendment 5 (protocol version 6.0) goes into effect will have baseline troponin I testing prior to treatment with AVXS-101, and will have troponin I testing in place of CK-MB. CK-MB will be collected from all other participants. (X): Months 2, 6, and 9 visits.
Troponin I[19]	X				X			X		X		(X)[16]	X	
~~Coagulation~~	~~X~~[3]	~~X~~[11]		~~X~~[15]	~~X~~	~~X~~	~~X~~	~~X~~				~~X~~	~~X~~	
~~Urinalysis~~	~~X~~[3]	~~X~~[11]		~~X~~[15]	~~X~~	~~X~~	~~X~~	~~X~~				~~X~~	~~X~~	
Virus Serology	(X)[3]													(X): Baseline procedure must be completed within 30 days of dosing.
Blood for diagnostic confirmation testing[7]	X													To be performed by central laboratory.

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
~~Visit #~~	~~1~~	~~2~~			~~3~~	~~4~~	~~5~~	~~6~~				~~Monthly (7-16)~~	~~Month 12/ EOS (17)~~	
~~# of Days/Months in~~ Study Day	-60 to -2	-1	1	2-3~~1~~	7	14	21	30	~~37~~4	60	~~51~~2	Monthly Through ~~Month~~M 1~~14~~	M~~onth~~1 ~~25~~/EOS	
Window					~~+/-~~ 2							~~+/-~~ 7		
Saliva, Urine, and Stool Samples (for viral shedding)		X		(X)~~13~~	(X)~~13~~	(X) ~~13~~		(X)~~13~~						(X): Sites participating in the viral shedding sub-study will collect 24-hour full volume samples for urine and feces through 24 and 48 hours. All other sites will collect singular urine and feces samples within 24 hours and 48 hours of dosing. Full urine and feces samples will be collected for patients ≥ 48 months who are no longer in diapers.
Baseline Screening of Biological Mother (Anti-AAV9 Ab)[8]	(X)~~3~~													Serum sample collected at screening for anti-AAV9 antibodies. (X): Baseline procedure must be completed within 30 days of dosing. (X)*: Additional sampling may be performed at subsequent time points if ELISpot value(s) continue to be elevated, based on further discussion with the ~~Principal Investigator~~PI and Medical Monitor.
Immunology Labs (Anti-AAV9/SMN)[8]	(X)~~3~~				X	X	X	(X)~~14~~						
Immunology Labs (IFN-γ T-cells)					X	X	X	(X)~~14~~						(X): Additional sampling may be performed at subsequent time points if ELISpot value(s) continue to be elevated, based on further discussion with the ~~Principal Investigator~~PI and Medical Monitor.
Prophylactic Prednisolone ~~dosing~~[9]		X	X	X	X	X	X	X	X	X	X			~~Dosing through Day 30, then follow taper schedule~~See Section 9.2.1. Daily dosing from 24 hours prior to scheduled AVXS-101 dose and continued as per protocol.
Study Product Administration [redacted]			X											

Study Interval	Baseline Screening	Vector (AVXS-101) Injection (Inpatient)			Follow-up (Outpatient)									Notes
~~Visit #~~	~~1~~	~~2~~			~~3~~	~~4~~	~~5~~	~~6~~				~~Monthly (7-16)~~	~~Month 12/ EOS (17)~~	
~~# of Days/Months in~~ Study Day	-60 to -2	-1	1	2-3~~[1]~~	7	14	21	30	~~374~~ 4	60	~~517~~ 2	Monthly Through ~~Month~~M 1~~14~~	M~~onth~~1 ~~25~~/EOS	
Window					~~+/-~~ 2							~~+/-~~ 7		
[redacted]														
Photograph Injection Site			X		X	X	X	X						
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	
Prior and Concomitant Medications	*To be collected from 2 weeks before study dosing until final study visit*													

AE = adverse event, ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK-MB = Creatinine kinase-MB (isozyme MB); ECG = electrocardiogram; ELISpot = Enzyme-linked ImmunoSpot; GGT = gamma glutamyl transferase; IFN-γ = interferon gamma; PI = Principal Investigator;SoA = Schedule of Assessments;

~~1 Patients to remain inpatient 48 hours post-dose for observation and AE monitoring.~~
~~2 To be performed at the Month 3, 6, and 9 visits.~~
~~3 Baseline procedure must be completed within 30 days of dosing.~~
~~4 Vital signs will include blood pressure, respiratory rate, pulse, and axillary temperature. Vitals including blood pressure, respiratory rate, pulse axillary temperature, pulse oximetry and heart rate will be monitored and recorded every 15 minutes (+/- 5 minutes) for four hours and every hour (+/- 15 minutes) for 24 hours following the AVXS-101 dosing procedure.~~
~~5 ECGs extracted from Holter by central reader in triplicate at the following time points: pre-dose, 2h, 4h, 6h, 8h, 12h, 24h, 36h, and 48h during the patient's inpatient stay.~~
~~6 Bayley III gross and fine motor sections will be performed at screening and monthly through 12 months.~~
~~7 To be performed by central laboratory.~~
~~8 Serum sample collected at screening for anti-AAV9 antibodies.~~
~~9 Prednisolone dosing through Day 30, then follow taper schedule.~~
~~10 Hammersmith Functional Motor Scale- Expanded will be conducted for patients ≥ 24 months of age; patients < 24 months of age at study entry will begin HFMS-E at such time that 24 months of age is reached.~~
~~11 Laboratory samples collected prior to dosing are to be processed locally.~~
~~12 Medical history collected at baseline; review of systems conducted at each study visit.~~
~~13 Sites participating in the viral shedding sub-study will collect 24-hour full volume samples for urine and feces through 24 and 48 hours. All other sites will collect singular urine and feces samples within 24 hours and 48 hours of dosing. Full urine and feces samples will be collected for patients ≥48 months who are no longer in diapers.~~

14 Additional sampling may be performed at subsequent time points if ELISpot value(s) continue to be elevated, based on further discussion with the Principal Investigator and Medical Monitor.
15 Laboratory samples collected on Day 2.
16 CK-MB or Troponin I sample will be collected at the following visits: Month 2 (Day 60), 6, 9.
17 Months 2, 3, 6, 9.
18 Irrespective of study schedule, after signing the updated informed consent, all patients enrolled prior to the Amendment 5 version of the protocol should have ECG, 24-hour Holter monitoring, and echocardiogram done at the next scheduled visit and then in conformity with the study schedule of assessments.
19 Troponin I will be measured instead of CK-MB in new patients who are screened and enrolled after amendment 5 (protocol version 6.0) goes into effect. Participants who have been screened and enrolled but who have not yet received gene replacement therapy (visit #2) at the time that amendment 5 (protocol version 6.0) goes into effect will have baseline troponin I testing prior to treatment with AVXS-101, and will have troponin I testing in place of CK-MB. CK-MB will be collected from all other participants.
20 Includes measuring head circumference.

Rationale for change

Reorganized section to include Study Design figure and SoA table in Section 7.1.

Updated study design to include follow-up for 15 months. Updated schedule of assessments to include Day 44 and Day 72 visits, and to clarify timing of visits. Footnotes moved to comments column for better readability. Updated to provide additional clarification on the dose level of dose C and the timing of the long-term follow-up study and the DSMB scheduling.

Section 7.2 - Section 7.5

Moved sections below Figure 4, Study Design and Table 3, Schedule of Assessments

Rationale for change

Moved sections to more appropriate location.

Section 8.1 Patient Inclusion Criteria

6. Be up-to-date on childhood vaccines~~. Seasonal vaccinations~~ that include palivizumab prophylaxis (also known as Synagis) to prevent RSV infections in accordance with the recommendations of the American Academy of Pediatrics (AAP 2009)

Rationale for change

Updated to clarify the wording on patient age range and vaccinations.

Section 8.2 Patient Exclusion Criteria

19. **Clinically significant abnormal laboratory values (GGT, ALT, and AST, or total bilirubin > 2 × ULN, creatinine ≥ 1.0 mg/dL, hemoglobin [Hgb] < 8 or > 18 g/dL; white blood cell [WBC] > 20,000 per cmm) prior to gene replacement therapy. Patients with an elevated bilirubin level that is unequivocally the result of neonatal jaundice shall not be excluded.**~~Abnormal laboratory values considered to be clinically significant (INR > 1.4), GGT > 3X ULN, Bilirubin ≥ 3.0 mg/dL, Creatinine ≥ 1.0 mg/dL, Hgb <8 or >18 g/Dl; WBC > 20,000 per cmm) prior to study dosing~~

Rationale for change

Updated text to include more stringent liver function tests further to the recommendations following the acute liver failure case reported in the US Managed Assess Program.

Section 8.3 Patient Withdrawal Criteria and Discontinuation

~~Patients may be discontinued from the study for the following reasons:~~

- ~~Development of unacceptable toxicity, defined as the occurrence of any unanticipated CTCAE Grade 3 or higher Adverse Event/toxicity that is possibly, probably, or definitely related to the gene replacement therapy, and is associated with clinical symptoms and/or requires medical treatment~~

Patients meeting the following criteria will be withdrawn:

- Death
  - Autopsies will be requested of any patients, with the exception of untreated patients, that expire following participation in a gene transfer study~~; see Autopsy Plan in Appendix 2~~
- Failure to comply with protocol-required visits or study procedures for 3 or more consecutive visits that are not rescheduled, unless due to hospitalization
- Parent(s)/legal guardian(s) withdraws consent
- Investigator discretion

Early termination procedures should be completed within 14 days for any patient who prematurely discontinues the study for any reason. **Patients who terminate the study early for reasons other than death will be offered enrollment in a long-term follow-up study.**

Rationale for change

Updated patient withdrawal and discontinuation criteria and clarified that patients will be offered to continue on the long-term follow-up study.

Section 9.1 Description of Study Product

Table 4 Investigational Product

	Investigational Product
Product Name	AVXS-101
Unit Dose	6.0×10^{13} vg (Dose A) 1.2×10^{14} vg (Dose B) ~~No more than~~ 2.4×10^{14} vg (Dose C)
Route of Administration	Intrathecal Injection
Physical Description	Once thawed, AVXS-101 is a clear to slightly opaque, colorless to faint white solution, free of visible particulates

Rationale for change

Updated to provide additional clarification on the dose level of Dose C.

Section 9.2.1 Prophylactic Administration of Prednisolone

In an attempt to dampen the host immune response to the AAV based therapy, patients will receive prophylactic prednisolone (glucocorticoid) (approximately 1 mg/kg/day) 24 hours prior to AVXS-101 dosing. Treatment will continue for approximately 30 days in accord with the following treatment guideline:

- Until at least 30 days post-~~infusion~~**injection**: 1 mg/kg/day
- Weeks 5 and 6 **post-injection**: 0.5 mg/kg/day

- Weeks 7 and 8 **post-injection**: 0.25 mg/kg/day
- Week 9 **post-injection** prednisolone discontinued

Liver function testing should guide each step of the taper, and liver function tests should be checked prior to prednisolone discontinuation. If the GGT, AST, or ALT values are ≥ 2 × ULN, then the present dose of prednisolone will be adjusted as needed until the GGT, AST, and ALT values decrease below threshold, at which point the taper may continue. Liver function tests should also be checked approximately 2 weeks after the taper has concluded and prednisolone has been discontinued to evaluate for rebound elevation of GGT, AST, or ALT levels. Variance from these recommendations will be at the discretion of the Investigator based on potential safety issues for each patient. If another glucocorticoid is used in place of prednisolone by the Investigator, similar considerations should be taken into account after 30 days and tapered as appropriate and at the discretion of the Investigator. ~~If the aspartate aminotransferase (AST) or alanine aminotransferase (ALT) values are > 2 X upper limit of normal (ULN), or if T-cell response is ≥ 100 SFC/106 PBMCs after 30 days of treatment, the dose of prednisolone will be maintained until the AST and ALT values decrease below threshold. If T-cell response continues past Day 60, investigator discretion should be used considering risk-benefit for maintaining prednisolone. Variance from these recommendations will be at the discretion of the investigator based on potential safety issues for each patient.~~

Rationale for change

Updated further to the recommendations following the acute liver failure case reported in the US Managed Assess Program and to clarify the wording

Section 9.2.3 Vaccinations

Where feasible, the vaccination schedule should be adjusted appropriately to accommodate prednisolone use. When avoiding vaccination while on steroids represents an undue delay or interruption of a vaccination schedule, vaccination should continue at the discretion and judgment of the treating physician given 1) the importance of maintaining childhood vaccination in this population and 2) the published literature that indicates that vaccination while on steroid doses 1 mg/kg/day or below is safe and effective [Mendell, 2017; Kroger, 2018].

Rationale for change

Added section to include guidance on vaccinations.

Section 10 Study Product Materials and Management

AVXS-101 was manufactured in accordance with current Good Manufacturing Practice (cGMP). ~~Investigational product accountability logs will be maintained by the clinical pharmacy.~~

Rationale for change

Deleted sentence for accuracy.

Section 10.4 Study Product Storage and Destruction

Rationale for change

Administrative update to section heading.

Section 10.6 Study Product Administration

Patients will receive AVXS-101 IT injection 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. **After p**~~P~~atients are admitted, ~~and~~ vitals **will be** monitored **immediately after dosing and** every 15 (~~±~~ 5) minutes ~~from dosing~~ for four hours and every hour (~~±~~ 15 minutes) for 24 hours following the AVXS-101 dosing procedure.

Rationale for change

Updated text to clarify timing of vital sign monitoring.

Section 10.6.1 Post-Administration Procedures

Patients will be kept in the PICU patient room or other appropriate setting (e.g., interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management for 48 hours for closer monitoring of mental status. 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, gloves, masks, glasses, and closed-toe shoes. Patients' families will be provided standardized, IRB-approved handouts regarding monitoring for mental status changes which includes monitoring for **severe headache,** fever, irritability, neck pain **or stiffness,** light sensitivity, ~~and~~ vomiting**, and lethargy (tiredness)**. Patients may be discharged from the hospital when the following criteria are met:

Rationale for change

Updated text to match the symptoms listed in the patient-facing letter.

Section 10.7 Dose Escalation

~~The investigators~~**AveXis** will confer with the DSMB**/DMC** on all Grade III or higher AEs within **approximately** 48 hours of awareness that are possibly, probably, or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB**/DMC** during quarterly meetings; following enrollment of the first three ~~(3)~~ patients ≥6 months and < 24 months of age at the time of dosing and based upon the available safety data a decision will be made whether to: a) stop due to toxicity, or b) proceed to Cohort 2 using Dose B.

Should the determination be made to advance to Cohort 2 (Dose B), again there will be at least a 4-week interval between dosing of the first three ~~(3)~~ patients < 60 months of age at the time of dosing within the cohort. Based on the available safety data from the first three ~~(3)~~ Cohort 2

patients and all of the Cohort 1 patients, the DSMB/**DMC** may decide and document during quarterly meetings that further 4-week intervals between patients dosing is unnecessary. AveXis~~, Inc.~~ will take this recommendation into consideration and will make the final determination whether to persist with 4-week intervals between patients dosing going forward. The decision will be communicated to sites and IRBs in a formal sponsor letter. ~~The investigators~~**AveXis** will confer with the DSMB/**DMC** on all Grade III or higher AEs within **approximately** 48 hours that are possibly, probably, or definitely related to the study agent before continuing enrollment. Safety data will be reviewed by the DSMB/**DMC** during quarterly meetings; following enrollment of the first six ~~(6)~~ patients and based upon available safety data a decision will be made whether to a) stop due to toxicity or b) continue to enroll an additional 21 patients until ~~twelve (~~12~~)~~ patients ≥6 months and < 24 months of age at time of dosing and ~~twelve (~~12~~)~~ patients > 24 < 60 months of age at time of dosing have received Dose B.

Based upon an ongoing assessment of safety and efficacy data from patients treated with the 1.2×10^{14} vg dose, an option for testing of a third dose (Dose C), will be considered. If, based on all available data, this is judged to be safe and necessary, three ~~(3)~~ patients < 60 months of age will receive Dose C ~~which will be no greater than~~ 2.4×10^{14} vg administered IT. A meeting of the DSMB/**DMC** will be called to obtain a recommendation on the safety of escalating to a higher dose prior to proceeding. If a decision is made to proceed to testing a higher dose, there will again be a four-week interval between dosing of the first three patients receiving Dose C, as in Cohorts 1 and 2. Safety data will be reviewed by the DSMB/**DMC** during quarterly meetings. Following enrollment of the first three ~~(3)~~ Dose C patients and based upon available safety data, the DSMB/**DMC** will be consulted and a decision will be made whether to: a) stop dosing Dose C due to safety concern, or b) continue to enroll an additional 21 patients until there are a total of ~~twelve (~~12~~)~~ patients ≥6 months and < 24 months and ~~twelve (~~12~~)~~ patients ≥ 24 and < 60 months that have received Dose C.

Rationale for change

Updated text to clarify the DSMB/DMC meeting and timing, and the dose level of Dose C.

Section 11.1 ~~Physical Therapy~~Physical ~~Clinic~~ Assessments

Rationale for change

Section title changed for accuracy.

Section 11.1.1 Hammersmith Functional Motor Scale-Expanded

The HFMSE will be administered by a **qualified** ~~physical therapist~~**clinical evaluator (e.g., licensed** physical **or occupational** therapist**, or national equivalent)** in accord~~ance~~ with Table 3 ~~within 30 days of dosing and monthly through twelve (12) fifteen (15) months~~ for all patients ≥ 24 months of age. Patients < 24 months of age at time of dosing will begin having ~~Hammersmith Fuctional Motor Scale Expanded~~**HFMSE** assessments at such time that 24 months of age is reached.

Rationale for change

Updated to clarify the roles of the assessors.

Section 11.1.2 Bayley Scales of Infant and Toddler Development

Bayley Scales of Infant and Toddler Development ©version 3 is a standardized, norm-referenced infant assessment **and will be administered by a qualified clinical evaluator (e.g., licensed physical or occupational therapist, or national equivalent).** The gross and fine motor subtests will be completed ~~within 30 days before dosing at baseline~~ **in accordance with** Table 3 ~~and then monthly through Month 12~~**.** See **AVXS-101-CL-102** Physical **Assessments** ~~Therapy~~ Manual.

Rationale for change

Updated to clarify the roles of the assessors.

Section 11.2 Motor Milestone Development Survey

The achievement of significant motor milestones will be assessed by the **qualified** ~~physical therapist~~**clinical evaluator (e.g., licensed** physical **or occupational** therapist**, or national equivalent)** using a standard Motor Milestone Development Survey shown in Table 5 with definitions of each milestone driven by **the** Bayley Scales **of Infant and Toddler Development** (see **AVXS-101-CL-102** Physical ~~Therapy~~ **Assessments** Manual). The ~~physical therapist~~**clinical evaluator** will record whether the patient has attained each of the milestones on the Motor Milestone Development Survey in accordance with Table 3. Once observed, a motor milestone is considered attained. The date of attainment of each motor milestone will be determined by the date of the visit in which the milestone is observed.

During the screening visit, the ~~physical therapist~~**clinical evaluator** will complete an assessment of baseline milestone achievement in accordance with Table 3; this assessment must be recorded on video and the findings must be documented in the source.

As the Bayley Scales do not necessarily require the child to repeat previously attained milestones, it is essential that each milestone be captured on video. Development milestone assessment sessions will be videotaped in accord with the AVXS-101-CL-102 Video**taping** Manual.

Rationale for change

Updated to clarify the roles of the assessors.

Section 11.3 Video Evidence

~~Physical therapy~~ **Clinical** assessments required at each study visit will be videotaped in an effort to produce compelling, demonstrable, documented evidence of efficacy, as determined by changes in functional abilities~~. Parent(s)/legal guardian(s) may also share home videos demonstrating achievement of functional abilities with the study site.~~ AveXis will provide a secure and confidential upload process for transfer and storage of the videos from investigational sites to a contracted third-party vendor that will compile and arrange videos as per AveXis requirements. Any/all videos received at AveXis or the contracted vendor will be treated as confidential study data and will be the sole property of AveXis. AveXis and the contracted vendor will provide this secure, encrypted transfer and storage solution to properly protect the identities of patients/families on the videos, which may be shared with regulatory agencies

and/or the medical community **and/or in appropriate venues to discuss the results of this clinical trial**.

Videos will be provided to an independent, centralized reviewer for unbiased assessment of milestone achievement. The independent **central** reviewer will ~~use the Motor Milestone Development Survey to~~ document whether the video displays evidence of having achieved each ~~motor~~ **developmental** milestone. The date of motor milestone achievement will be computed as the earliest **date on which video evidence demonstrates the achievement** of the ~~video dates in which achievement of the~~**specified developmental** milestone~~has been demonstrated.~~

Additionally, the parent(s)/legal guardian(s) may submit additional videos demonstrating achievement of developmental milestones at any time during the trial. These videos will be handled in the same manner in which the trial-derived videos are handled.

Rationale for change

Updated to clarify the roles and responsibilities of the assessors.

Section 12.3 Vital Signs

Paragraph 3

~~At Visit 2~~**After patients are admitted**, vitals including blood pressure, respiratory rate, pulse axillary temperature, pulse oximetry and heart rate will be monitored and recorded every 15 minutes (± 5 minutes) **after dosing** for four hours and every hour (± 15 minutes) for 24 hours following the AVXS-101 dosing procedure.

Rationale for change

Updated text to clarify timing of vital signs assessments.

Section 12.6 Vaccine Recommendations

Patients are encouraged to follow all routinely scheduled immunizations as recommended by the Center for Disease Control (CDC~~). Seasonal vaccinations that include~~**including** palivizumab prophylaxis (also known as Synagis) to prevent ~~respiratory syncytial virus (~~RSV~~)~~ infections ~~are also recommended~~ in accordance with **the recommendations of the** American Academy of Pediatrics (AAP 2009).

Rationale for change

Added section to include requirement for vaccination per palivizumab prophylaxis

Section 12.7 Electrocardiogram ~~(ECG)~~

A 12-lead ECG will be performed ~~at screening/baseline, Day 1, Day 2, Day 3, Month 3, Month 6, Month 9, and Month 12~~**as specified in the SoA** ~~(or Early Termination)~~ (Table 3**).** For patients enrolled in the study prior to Amendment 5 and irrespective of study schedule, after signing an updated informed consent, all patients should have a 12-lead ECG at their next scheduled visit and then in conformity with the ~~Schedule of Assessments~~**SoA** (Table 3).

The ECG will be interpreted locally by a cardiologist or designee for immediate safety evaluation. The ECG tracings or ECG machine data will **also** be collected for centralized review **and interpretation** by a cardiologist.

A 12-Lead ECG will be performed (concurrent with Holter Monitor) ~~on the day of gene delivery and on Day 2 and Day 3 post-gene delivery~~**as specified in the SoA (Table 3)**. Additional electrophysiological monitoring will be at the discretion of the investigator as per local institutional guidelines.

Rationale for change

Updated to clarify timing and interpretation of the assessment and deleted text to reduce redundancy within the protocol.

Section 12.8 12-Lead Holter Monitor

Twenty-four-hour Holter monitoring will also be performed ~~at screening and Months 1, 2, 3, 6, 9 and 12 visits (or Early Termination).~~**as specified in the SoA (Table 3)**. For patients enrolled in the study prior to Amendment 5 and irrespective of study schedule, after signing an updated informed consent, patients should have a 24-hour Holter monitor at their next scheduled visit and then in conformity with the ~~schedule of assessments~~**SoA**.

Holter monitors will be provided to study sites along with a dedicated laptop for uploading the data from the memory cards for centralized review and analysis by a cardiologist within 24 hours of data upload. **AveXis**~~The sponsor physician~~ will be notified of any safety concerns from the centralized review ~~and the safety management plan followed for documenting and reporting of AE/Serious Adverse Events (SAEs)~~.

Rationale for change

Updated to reduce redundancy within the protocol and to clarify who will be notified with safety concerns.

Section 12.9 Echocardiogram

An echocardiogram will be performed ~~at screening/baseline, and at the Month 3, Month 6, Month 9, and Month 12 Visits~~**as specified in the SoA** ~~(or Early Termination)~~ **(Table 3)**. For patients enrolled in the study prior to Amendment 5 and irrespective of study schedule, after signing an updated informed consent, all patients should have a transthoracic echocardiogram at their next scheduled visit and then in conformity with the **SoA (**Table 3.**).**

The echocardiogram will be interpreted locally by a cardiologist or designee for immediate safety evaluation. The echocardiograms will also be collected for centralized review and interpretation by a cardiologist.

Rationale for change

Updated to clarify timing and process of interpretation of the assessment and deleted text to reduce redundancy within the protocol.

Section 12.11 Pulmonary Exam

Pulmonary examinations will be performed by a pulmonologist (or appropriate individual as per standard institutional practice)~~Patients will be assessed by a pulmonologist~~ at the time points specified in Table 3. **Prior to trial entry, a pulmonologist (or appropriate individual as per standard institutional practice) will review and document ventilator usage in the 2 weeks prior to screening.** ~~and~~

Patients may be fitted with a non-invasive positive pressure ventilator (e.g., BiPAP) at the discretion of the pulmonologist and/or investigator. Non-invasive ventilatory support equipment will be provided by AveXis, Inc. through a third-party vendor **if not covered by the patient's insurance.**

Should the patient require non-invasive ventilator support at any time during the trial, the equipment to be provided must be approved by AveXis in order to ensure ability to upload usage data.

Patients requiring non-invasive ventilatory support will be asked to bring the machine to each study visit such that the study staff can remove ~~an SD card~~**storage device** which ~~records~~ **captures** actual usage data. **The hours per day usage data for each day between visits will be extracted with software provided by the device manufacturer into a format that will be transferred/transcribed to the clinical database.**~~This usage data will be transferred to the clinical database.~~

~~Patients requiring non-invasive ventilatory support will be asked to remove the SD card and ship it to the study site in instances of missed study visits.~~

Rationale for change

Updated with administrative changes and to clarify the roles of the assessors and to clarify procedures.

Section 12.13 Photographs of Injection Site

Photographs will be taken of the injection site ~~through Day 30~~ at the time points specified in Table 3 to monitor healing of the injection wound. **Day 1 injection site photograph will be performed prior to the start of gene replacement therapy injection.** AveXis, Inc. will provide a secure and confidential upload process for transfer and storage of the photographs from the investigative sites to a contracted third-party vendor that will compile and arrange photographs as per AveXis, Inc. requirements. Any/all photographs received at AveXis, Inc. or the contracted vendor will be treated as confidential study data and will be the sole property of AveXis, Inc. AveXis, Inc. and the contracted vendor will provide this secure, encrypted transfer and storage solution to properly protect the identities of patients/families in the photographs, which may be shared with regulatory agencies, ~~and/or~~ the medical community**, and/or in appropriate venues to discuss the results of this clinical trial.**

Rationale for change

Updated with administrative changes to reflect the timing of the assessment and deleted text to reduce redundancy within the protocol.

Section 12.14 Laboratory Assessments

Table 7 Total Blood Volume

Visit	Tests[1, 3]	Total Volume (mL)[2]
Screening	Hematology, chemistry/CK-MB or Troponin I, coagulation, virus serology, immunology sample (AAV9/SMN Ab only), diagnostic confirmation sample	19.3-19.6 ~~mL~~
Day -1	Hematology, chemistry, coagulation, capillary blood gas	6.0 ~~mL~~
Day 2	Hematology, chemistry, coagulation, capillary blood gas	6.0 ~~mL~~
Day 7	Hematology, chemistry/CK-MB or Troponin I, coagulation, immunology sample	10.0-12.3[+] ~~mL~~
Day 14	Hematology, chemistry, coagulation immunology sample	9.0-11.0[+] ~~mL~~
Day 21	Hematology, chemistry, coagulation immunology sample	9.0-11.0[+] ~~mL~~
Day 30	Hematology, chemistry/CK-MB or Troponin I, coagulation, immunology sample	11.0-12.3[+] ~~mL~~
Day 44	~~Hematology~~, ~~chemistry~~**Chemistry**	**1.3**
Day 60	**Hematology, chemistry/CK-MB or Troponin I, coagulation**	**6.0-6.3**
Day 72	~~Hematology~~, ~~chemistry~~**Chemistry**	**1.3**
~~Month 2~~	~~Hematology, chemistry/CK-MB[3] or Troponin I, coagulation~~	~~6.0-6.3 mL~~
Month 3	Hematology, chemistry, coagulation	5 ~~mL~~
Month 4	Hematology, chemistry, coagulation	5 ~~mL~~
Month 5	Hematology, chemistry, coagulation	5 ~~mL~~
Month 6	Hematology, chemistry/CK-MB[3] or Troponin I, coagulation	6.0-6.3 ~~mL~~
Month 7	Hematology, chemistry, coagulation	5 ~~mL~~
Month 8	Hematology, chemistry, coagulation	5 ~~mL~~
Month 9	Hematology, chemistry/CK-MB[3] or Troponin I, coagulation	6.0-6.3 ~~mL~~
Month 10	Hematology, chemistry, coagulation	5 ~~mL~~
Month 11	Hematology, chemistry, coagulation	5 ~~mL~~
Month 12	**Hematology, chemistry/CK-MB or Troponin I, coagulation**	**6.0-6.3~~5~~**
Last Study Visit (Month 15~~2~~)[4]	Hematology, chemistry/CK-MB[3] or Troponin I, coagulation	6.0-6.3 ~~mL~~
Total Volume for Study 1-Year Duration		**13~~7.95~~-14~~7.33~~~~7.1 mL~~[3]**

Immunology = Serum antibody to AAV9 and SMN, IFN-γ ELISpot to detect T-cell responses to AAV9 and SMN

1 Immunology sample requires 4-6 mL of whole blood

2 Maximum total blood volume specified for each visit; in cases where tests can be combined, lower blood volumes may be sufficient. Positive serology samples at screening and elevated T-cell responses at Day 30 may require additional surveillance samples not reflected in the table.

3 Troponin I will be measured instead of CK-MB in new patients who are screened and enrolled after amendment 5 (protocol version 6.0) goes into effect. Participants who have been screened and enrolled but who have not yet received gene replacement therapy (visit #2) at the time that amendment 5 (protocol version 6.0) goes into effect will have baseline troponin I testing prior to treatment with AVXS-101, and will have troponin I testing in place of CK-MB. CK-MB will be collected from all other participants.

4 **Only patients enrolled in Cohort 3 will have a month 15 visit. Patients enrolled in Cohorts 1 and 2 will complete the study or have EOS visit at Month 12**

Rationale for change

Updated blood volume required for additional visits (Day 44, Day 72, Month 15) added for liver function tests and additional follow-up visits.

Section 12.14 Laboratory Assessments

If there is not sufficient blood volume to include the genetic reconfirmation testing sample at the screening visit, the patient must return before ~~Visit 2.~~**the inpatient period**. All patients must have genetic reconfirmation testing completed.

Rationale

Visits are no longer numbered. Updated for SoA clarity.

Section 12.14.1 Hematology

Hematology analysis will include a CBC with differential and platelet count with smear. Samples will be collected and shipped in accord with the laboratory manual provided by the central laboratory **as per the time points specified in Table 3**.

Rationale

To reduce redundancy and direct to SoA.

Section 12.14.2 Serum Chemistry

Samples will be collected and shipped in accord with the laboratory manual provided by the central laboratory **as per the time points specified in Table 3.**

CK-MB or Troponin I will be collected **as specified in the SoA**at screening, Day 7, Day 30, Day 60 and at Months 6, 9, and 12/End of Study.

Rationale

To reduce redundancy and direct to SoA.

Section 12.14.7.1 ELISA: Anti-AAV9-Ab

Blood samples will be collected and shipped to the central laboratory in accord with the laboratory manual to test for serum antibodies to AAV9 ~~at screening and~~ as per the timepoints specified in Table 3.

Rationale

Updated to reduce redundancy and direct to SoA.

Section 12.14.8 Baseline Screening of Biological Mother

There is potential that the **biological** mother of the enrolled patient may have pre-existing antibodies to AAV9 that may be transferred to the patient via placental transfer in utero or theoretically through breast milk. Informed consent will be requested from the **biological** mother of the patient to screen the **biological** mother for circulating antibodies to AAV9. Once informed consent has been obtained, the **biological** mother will have her blood drawn from a peripheral vein and shipped to the central laboratory for screening of anti-AAV9 antibodies.

If AAV9 antibodies are identified, the investigator should discuss with the **biological** mother whether to continue or to stop breastfeeding.

Rationale for change

Updated text to reflect that this will only be performed on the biological mother.

Section 12.14.10 Saliva, Urine, and Stool Collection

Studies have shown that some vector can be excreted from the body for up to a few weeks after injection; this is called "viral shedding". Vector shedding can be found in the blood, urine, saliva, and stool for up to a week 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, IRB-approved instructions should be provided to patient families and care givers regarding use of protective gloves if/when coming into direct contact with patient bodily fluids and/or waste as well as good hand-hygiene for a minimum of ~~two~~ **four** weeks after the injection.

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

Saliva, urine, and stool samples will be collected in accord with the laboratory manual for viral shedding studies in accord with Table 3 ~~including 24 hours and 48 post- doses~~. Patients at all sites ≥ 48 months **of age** who are no longer in diapers will provide full volume urine and full volume feces samples ~~at Day 7, Day 14, and Day 30~~**at the timepoints specified in the SoA (Table 3)** for at least one void and one defecation. Samples will be prepared as per the laboratory manual, stored in a -80 freezer, and shipped to the central laboratory in accord with the laboratory manual.

Rationale for change

Updated to reduce redundancy and direct to SoA.

Section 13.1.1 Adverse Event ~~(AE)~~

All AEs that occur after any patient has received treatment, during treatment, or up through the last study visit, whether or not they are related to the study, must be recorded on forms provided by **Syneos Health, formerly** INC **(a Contract Research Organization responsible for managing specific aspects of the study, as designated by AveXis).**

Unanticipated CTCAE Grade 3 or higher AEs that are possibly, probably, or definitely related to ~~the product~~ **gene replacement therapy** must be reported within 24 hours to ~~Sponsor~~ **Avexis** and/or designee ~~as per trial safety management plan~~ to ensure timely escalation to the DSMB/Data Monitoring Committee (DMC).

Rationale for change

Administrative change.

Section 13.1.1 Serious Adverse Event

All SAEs that occur after any patient has been enrolled, before treatment, during treatment, or up through the last study visit, whether or not they are related to the study, must be recorded on forms provided by **Syneos Health**~~INC~~.

Rationale for change

Administrative change.

Section 13.1.3 Other Adverse Event ~~(OAE)~~

The following specific treatment-emergent AE of special interest (AESI), which may be searched using Standardized MedDRA queries, will be summarized:

- **Elevated liver enzymes**

Grade 3 or higher elevated liver enzyme events related to AVXS-101 must be collected and recorded on forms provided by the Contract Research Organization. These events should be reported within 24 hours of occurrence whether or not they are deemed to be an SAE.

Other adverse events (OAEs) ~~may~~ **could** be identified by the Drug Safety Physician and if applicable also by the ~~Medical Monitor~~**Clinical Trial Team Physician** ~~and/or Drug Safety Monitor (DSM)~~ during the evaluation of safety data for the Clinical Study Report. **Examples of AESIS include hepatotoxicity, cardiac toxicity and thrombocytopenia.** Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the patient from the study, will be classified as OAEs. For each OAE, a narrative may be written and included in the Clinical Study Report.

Rationale for change

Updating and clarifying the requirements for reporting elevated liver enzymes accordingly. Provided further clarification on other adverse events reporting in the Clinical Trial Report.

Section 13.3 Recording Adverse Events

AEs spontaneously reported by the patient or family and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. However, abnormal values that constitute an SAE or lead to discontinuation of administration of study product must be reported and recorded as an AE. Information about AEs will be collected from the time of IT injection until the end of the study. SAE information

will be collected from signing of consent form until the last study visit. The AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the onset (date and time (if start date during ~~Visit 2~~**inpatient period**)), resolution (date and time (if start date during ~~Visit 2~~**inpatient period**)), intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study.

Rationale for change

Administrative change

Section 13.4 Reporting Serious Adverse Events

All SAEs (related and unrelated) will be recorded from signing of consent form through the last study visit. Any SAEs considered possibly, probably, or definitely related to the investigational product and discovered by the Investigator at any time after the study should be reported. All SAEs must be reported to AveXis via **Syneos Health**~~INC~~ within 24 hours of the first awareness of the event. The Investigator must complete, sign and date the SAE pages, verify the accuracy of the information recorded on the SAE pages with the corresponding source documents, and send a copy by fax or email to **Syneos Health**~~INC~~.

Additional follow-up information, if required or available, should all be faxed or emailed to **Syneos Health**~~INC~~ within 24 hours of receipt and this should be completed on a follow-up SAE form, and placed with the original SAE information and kept with the appropriate section of the eCRF and/or study file.

Rationale for change

Administrative change

Section 13.5 Expedited Safety Reporting to Regulatory Authorities

All unexpected, serious, drug-related events which are fatal or life-threatening will be reported to relevant regulatory authorities in an expeditious manner. All other unexpected, serious, drug-related events will be reported to relevant regulatory authorities within 15 days.

Rationale for change

Added language to be consistent with protocol template across all studies.

Section 14.6.1 Observation Period for Efficacy

Efficacy comparisons will be made once all patients have either completed 1~~2~~**5** months of follow- up or withdrawn prior to 1~~2~~**5** months of follow-up. **The primary analysis of efficacy data will be based on data collected through the 12-month time point; a supportive analysis of efficacy data will be based on data collected through the 15-month time point.**

Rationale for change

Included follow-up through 15 months.

Section 14.6.2.1 Primary Efficacy Endpoint: patients ≥6 months and < 24 months at time of dosing

~~For the cohort regarded as having received the optimal dose, t~~The proportion of patients ≥6 months and < 24 months of age at time of dosing achieving the ability to stand without support up to the 12-month study visit will be compared between AVXS-101 and natural history control groups using a two-sample 2-sided superiority Fisher exact test. A 95% exact conditional confidence interval for the difference in response rates will be computed.

The primary analyses will be based on the Full Analysis Set for patients ≥6 months and < 24 months of age at the time of dosing.

A sensitivity analysis will be conducted by repeating each primary efficacy analysis on the Efficacy Completers Analysis Set for patients ≥ 6 months and < 24 months of age at the time of dosing.

A supportive analysis will be conducted by repeating the analysis of the primary efficacy endpoint for patients ≥ 6 months and < 24 months of age considering data through the 15-month visit.

Rationale for change

Included follow-up through 15 months.

Section 14.6.2.2 Primary Efficacy Endpoint: patients ≥ 24 months and < 60 months at time of dosing

A supportive analysis will be conducted by repeating the analysis of the primary efficacy endpoint for patients ≥ 24 months of age and < 60 months of age considering data through the 15-month visit.

Rationale for change

Included follow-up through 15 months.

Section 14.6.3 Secondary Efficacy Endpoint

~~For the cohort regarded as having received the optimal dose, t~~The proportion of patients ≥6 months and < 24 months or ≥ 24 and < 60 months at time of dosing who achieve the ability to walk without assistance will be compared separately between AVXS-101 and natural history control groups using two-sample Fisher's 2-sided exact test. A 95% exact confidence interval for the differences in response rates will be computed.

A supportive analysis will be conducted by repeating the analysis of the secondary efficacy endpoint considering data through the 15-month visit.

Rationale for change

Included follow-up through 15 months.

[redacted]

Rationale for change

Included follow-up through 15 months.

Section 14.7 Safety Analysis

Safety will be assessed through monitoring AE reports and concomitant medication usage, and by conducting physical examinations, vital sign assessments, cardiovascular evaluations, and laboratory evaluations (chemistry, hematology, coagulation, immunology). Patients will be observed at the hospital for 48 hours post IT injection. Patients will return for follow-up visits ~~on Days 7, 14, 21, and 30. Patients will return monthly thereafter, following~~**in accordance with** the ~~Day 30 visit, for the first 12 months from dose administration~~**SoA**. Adverse events will be coded in accord with the most current version of the MedDRA coding dictionary.

Rationale for change

Administrative change to reduce redundancy within the protocol.

Section 15 DATA SAFETY MONITORING BOARD/DATA MONITORING COMMITTEE

The DSMB/DMC is an independent multidisciplinary group consisting of clinicians and a biostatistician that, collectively, have experience in the management of patients with SMA Type 1 and other diseases, and in the conduct and monitoring of randomized clinical studies with interim analyses. The DSMB/DMC will be chartered to oversee the safety of patients during the conduct of the trial and will act in an advisory capacity to AveXis. A detailed description of the DSMB/DMC, its role in this trial, and the timing of the scheduled reviews will be described in a DSMB/DMC Charter.

The DSMB/DMC will routinely convene on a quarterly basis to review emerging safety data from the trial. All available safety data from all enrolled patients will be included in such reviews, which include, but are not limited to, screen failures, enrollment status, data from safety parameters, all SAEs, and other AEs. Following each meeting, the DSMB/DMC will make a recommendation as to whether or not the accumulated safety data warrants a suspension or discontinuation of the trial, a modification to the trial, or

any additional comments or recommendations related to safety. The DSMB/DMC will prepare and provide minutes of their meetings to AveXis who will provide copies to the regulatory authorities as appropriate.

The DSMB/DMC will also convene on an ad hoc basis within approximately 48 hours should any patient experience an unanticipated CTCAE Grade 3 or higher AE/toxicity that is possibly, probably or definitely related to gene replacement therapy, and is associated with clinical symptoms and/or requires medical treatment. This includes any patient death, important clinical laboratory finding or any complication attributed to administration of gene replacement therapy. In such instances, the DSMB/DMC will review the safety information and provide its recommendation. Based upon the DSMB/DMC's review, the DSMB/DMC can recommend continuing enrollment, halting enrollment or early termination of the trial for safety reasons.

~~The DSMB will act in an advisory capacity to review patient safety and study progress for the clinical trial. Responsibilities of the DSMB are to:~~

- ~~Review the research protocol, informed consent documents and plans for data and safety monitoring~~
- ~~Evaluate the progress of the study, including periodic assessments of data quality and timeliness, patient recruitment, accrual and retention, patient risk versus benefit, study site performance, and other factors that can affect study outcome~~
- ~~Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on patient safety or the ethics of the study~~
- ~~Review study performance, make recommendations and assist in the resolution of problems reported by the investigators~~
- ~~Protect the safety of the study patients~~
- ~~Review safety data to determine whether to recommend dose escalation~~
- ~~Review quarterly safety data to determine safety signals or trends~~
- ~~Ensure the confidentiality of the study data and the results of monitoring~~

~~Assist by commenting on any problems with study conduct, enrollment, and sample size and/or data collection~~

Rationale for change

Administrative updates to DSMB membership, meetings and reporting.

Former Section 15.1 ~~DSMB Reporting and Meeting~~

~~Reports describing the status of the study will be prepared by AveXis, Inc. and sent to the DSMB at least quarterly, or at the DSMB's request. Reports will be submitted prior to a scheduled meeting for review by the DSMB.~~

~~Reports will include the following:~~

- ~~A brief narrative of the study status, including the target enrollment, current and projected time to completing enrollment. Any significant events and/or difficulties should be briefly described in this narrative~~
- ~~A brief narrative for each patient describing gender, age, race and ethnicity and other relevant demographic characteristics. The narrative for each patient should briefly describe his/her study status (i.e., dose level, visit number, AE information)~~
- ~~A timeline outlining the study progress relative to visit number for each patient, as well as time points for each SAE/Dose Limiting Toxicity. A total for AEs for each patient should be included~~
- ~~A summary of AEs by classification~~
- ~~A listing of AE details grouped by patient~~
- ~~A listing of SAE details grouped by patient~~
- ~~A listing of deaths~~
- ~~A summary of clinically significant laboratory test results~~
- ~~A listing of protocol deviations~~

Rationale for change

Administrative updates to DSMB membership, meetings and reporting.

Former Section 15.3 DSMB Membership

~~The DSMB membership will consist of persons completely independent of the investigative sites and sponsor who have no financial, scientific, or other conflicts of interest with the trial. The DSMB will include experts in or representatives of the fields of:~~

- ~~Neurology and Neuromuscular Diseases~~
- ~~Immunology~~
- ~~Gene Therapy~~
- ~~Spinal Muscular Atrophy Clinical Care~~
- ~~Clinical Research and Clinical Trials~~
- ~~Statistics (non-voting member)~~

~~Individuals invited to serve on the DSMB as either voting or non-voting members must disclose any potential conflicts of interest, whether real or perceived. Conflicts of interest can include professional, proprietary, and miscellaneous interests as described in 45 CFR Part 94. Potential conflicts that develop during a member's tenure on a DSMB must also be disclosed.~~

~~AveXis, Inc. will employ a third party (e.g. consulting group) to provide planning, organization, preparation, and oversight services for the DSMB meetings.~~

Rationale for change

Administrative updates to DSMB membership, meetings and reporting.

Section 18.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (please see Appendix 1) and are consistent with ICH/GCP, applicable regulatory requirements ~~and the AveXis, Inc.'s policies~~.

Rationale for Change

Administrative change.

Section 19.3 Retention of Records

The site will maintain **essential documents as required by ICH-** ~~**GCP**a Clinical Study Document Binder, which will be maintained at the study site. In this binder, there will be tabbed sections for study documents including the following: study personnel identification and signature list, patient / subject screening records, patient / subject roster (names omitted), protocol and amendments or administrative changes, FDA Form 1572 (if required), study staff Curricula Vitae, IRB/IEC documentation, an approved sample ICF, drug / product accountability records, correspondence, site monitoring reports, blank Data Documentation form, and lab accreditations and normal values.~~ The site must keep ~~this binder current and~~**these documents** available for review by the Sponsor, IRB/IEC, and/or regulatory bodies.

Rationale for Change

Administrative change.

Appendix 2 AUTOPSY PLAN

This was removed to be consistent across protocols. New sentence added to protocol: *An autopsy and tissue collection process will be in place for those patients who consent to autopsy/tissue collection for research purposes*.

Rationale for change

Details of autopsy included in manual not in protocol.