Protocol Number: AVXS-101-CL-304

Official Title: A Global Study of a Single, One-Time Dose of AVXS-101 Delivered to Infants with Genetically Diagnosed and Pre-symptomatic Spinal Muscular Atrophy with Multiple Copies of SMN2

NCT Number: NCT03505099

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AVXS-101
PROTOCOL AVXS-101-CL-304

IND Number:	15699	**EudraCT Number:**	2017-004087-35

Protocol Title: A Global Study of a Single, One-Time Dose of AVXS-101 Delivered to Infants with Genetically Diagnosed and Pre-symptomatic Spinal Muscular Atrophy with Multiple Copies of *SMN2*

Indication Studied: Spinal Muscular Atrophy

Sponsor Address: AveXis, Inc.
2275 Half Day Road
Bannockburn, IL 60015

Protocol Version/Date: 6.0 /Amendment 5/ 28 Jul 2020

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 study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

1. ADMINISTRATIVE INFORMATION

1.1. Approval

Representatives from AveXis:

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

- 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

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

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

[redacted]
Senior Medical Director, Translational Medicine
AveXis, Inc.
Date (ddMmmyyyy)

[redacted]
Associate Director, Therapeutic Lead
AveXis, Inc.
Date (ddMmmyyyy)

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

[redacted]
Senior Director, Biostatistics
AveXis, Inc.
Date (ddMmmyyyy)

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

1.2. Investigator's Agreement

I have received and read the Investigator's Brochure for AVXS-101. I have read the AVXS-101-CL-304 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 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 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 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)

1.3. Key Contact Information

Role in Study	Contact information
Responsible Physician	[redacted]. Senior Medical Director, Translational Medicine, AveXis, Inc., [redacted]
24-Hour Emergency Contact	Please see Study Contact List in ISF

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 delivered by Self-Complementary Adeno-Associated Virus Serotype 9 (AAV9)	
Title of Study: A Global Study of a Single, One-Time Dose of AVXS-101 Delivered to Infants with Genetically Diagnosed and Pre-Symptomatic Spinal Muscular Atrophy with Multiple Copies of *SMN2*	
Study Center(s): up to 32 global centers Japan is participating in this global study under a Japan-specific version of the AVXS-CL-101-304 protocol.	
Studied Period (years): Estimated date first patient enrolled: 1Q 2018 Estimated date last patient completed: *SMN2* 2 copies: 4Q 2020; 3 copies: 3Q 2021	**Phase of Development:** 3

Objectives:

Safety:

- Evaluate the safety of AVXS-101 through incidence of adverse events (AEs) and/or serious adverse events (SAEs)
- Evaluate the safety of AVXS-101 based on the change from baseline in clinical laboratory parameters

Efficacy objectives will be assessed independently for each cohort.

Efficacy for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

Primary:

- Assess the efficacy of AVXS-101 by demonstrating functional independent sitting [redacted] as defined by Bayley Scales of Infant and Toddler Development© Version 3 (BSIDv03) Gross Motor Subset Item #26 at any visit up to 18 months of age

Secondary:

- Determine the efficacy of AVXS-101 based on survival, defined as avoidance of death or the requirement of permanent ventilation in the absence of acute illness or perioperatively as assessed at 14 months of age
- Assess efficacy of AVXS-101 by demonstrating the ability to maintain weight at or above the third percentile without need for non-oral/mechanical feeding support at any visit up to 18 months of age

Exploratory:

- [redacted]

Efficacy for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

Primary:

- Assess the efficacy of AVXS-101 based on the proportion of patients achieving the ability to stand without support for at least 3 seconds as defined by BSIDv03 Gross Motor Subtest Item #40 at any visit up to 24 months of age

Secondary:

- Assess the efficacy of AVXS-101 by demonstrating the ability to walk alone [redacted], as defined by BSIDv03 Gross Motor Subtest Item #43, at any visit up to 24 months of age

Exploratory:

- [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
- [redacted]
  - [redacted]
  - [redacted]
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  - [redacted]
  - [redacted]
  - [redacted]
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- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

Methodology:

Phase 3, open-label, single-arm study of a single, one-time dose of AVXS-101 (gene replacement therapy) in patients with spinal muscular atrophy (SMA) who meet enrollment criteria and are genetically defined by bi-allelic deletion of survival motor neuron 1 gene (*SMN1*) with 2 or 3 copies of survival motor neuron 2 gene (*SMN2*). Patients with *SMN1* point mutations or the *SMN2* gene modifier mutation (c.859G>C) may enroll but will not be included in the efficacy analysis sets.

The study will enroll at least fourteen (14) patients with 2 copies of *SMN2* that meet the Intent-to-Treat (ITT) criteria and at least twelve (12) patients with 3 copies of *SMN2* that meet the ITT criteria. Patients in both cohorts must be ≤6 Weeks of age at the time of gene replacement therapy (Day 1).

The study includes a screening period, a gene replacement therapy period, and a follow-up period. During the screening period (Days -30 to -2), patients whose parent(s)/legal guardian(s) provide informed consent will undergo screening procedures to determine eligibility for study enrollment. Patients who meet the entry criteria will enter the in-patient gene replacement therapy period (Day -1 to Day 2). On Day -1, patients will be admitted to the hospital for pre-treatment baseline procedures. On Day 1, patients will receive a single, one-time intravenous (IV) infusion of AVXS-101 and will undergo in-patient safety monitoring for a minimum of 24 hours post infusion. Patients may be discharged 24 hours after the infusion, based on Investigator judgment. During the outpatient follow-up period (Days 3 to EOS) at 18 or 24 months of age, dependent upon respective *SMN2* copy number), patients will return at regularly scheduled intervals for efficacy and safety assessments until the EOS when the patient reaches 18 months of age (*SMN2* = 2) or 24 months of age (*SMN2* = 3). After the EOS visit, patients will be monitored for safety for 30 days. Additionally, patients will be invited to participate in a long-term follow up study conducted under a separate protocol.

After dosing, follow-up visits will be conducted according to the Schedule of Assessments. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose (Day 1). Visits following Day 72 are based on patient's date of birth. The EOS visit for patients with 2 and 3 copies of *SMN2* will be the 18 and 24 months old visits, respectively. However, safety monitoring will continue for 30 days after EOS visit. Any missed visit should be rescheduled as soon as possible within the window described in the Schedule of Assessments. All visits will be scheduled based on a 30-day month calendar.

In an attempt to dampen the host immune response to the adeno-associated virus (AAV) derived therapy, all patients will receive prophylactic prednisolone at approximately 2 mg/kg/day (or an equivalent dose of another glucocorticoid if prednisolone is unavailable or in the opinion of the investigator prednisolone is not tolerated) on Day -1, Day 1, and Day 2, and then 1 mg/kg/day starting on Day 3 and until at least 30 days post-AVXS-101 infusion. After 30 days post-AVXS-101 infusion, the dose of prednisolone can be tapered for patients whose gamma glutamyl transferase (GGT), alanine aminotransferase (ALT) values, and aspartate aminotransferase (AST) values are below the threshold of 2 × upper limit normal (ULN) in accordance with the following treatment guideline: taper from 1 mg/kg/Day to 0.5 mg/kg/Day during Weeks 5 and 6 post-AVXS-101 infusion, then taper to 0.25 mg/kg/Day during Weeks 7 and 8, and then discontinue prednisolone at Week 9.

If the GGT, AST, or ALT values are > 2 × ULN, the dose of prednisolone will be maintained until the GGT, AST, and ALT values decrease below threshold, at which point the taper may continue. 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.

Efficacy will be assessed by achievement of the key developmental milestone of functional independent sitting [redacted] (*SMN2* = 2) at any visit up to 18 months of age; and ability to stand without support for at least 3 seconds (*SMN2* = 3) at any visit up to 24 months of age. Additional developmental milestones will be assessed using the WHO-MGRS and BSIDv03. Safety will be assessed through monitoring AEs, concomitant medication usage, physical examinations, vital sign assessments, cardiac assessments, and laboratory evaluations. The primary efficacy analysis for each *SMN2* copy number cohort will be completed separately at such time that enrollment in the respective cohort is complete and the last patient has completed the EOS visit at the respective age or has discontinued (*SMN2* = 2, End of Study (EOS) = 18 months of age; *SMN2* = 3, EOS = 24 months of age).

Number of Patients (planned): A minimum of twenty-six (26) patients that meet ITT criteria

Diagnosis and Main Criteria for Inclusion: The study will enroll genetically diagnosed and pre-symptomatic spinal muscular atrophy patients with 2 or 3 copies of *SMN2.*

Inclusion Criteria:

All patients:

1. Age ≤6 Weeks (≤42 days) at time of dose
2. Ability to tolerate thin liquids as demonstrated through a formal bedside swallowing test
3. Compound muscle action potential (CMAP) ≥2 mV at Baseline; centralized review of CMAP data will be conducted
4. Gestational age of 35 to 42 weeks
5. Up-to-date on childhood vaccinations that include palivizumab prophylaxis (also known as Synagis) to prevent respiratory syncytial virus (RSV) infections are also recommended in accordance with the guidance of local health authorities.
6. Able and willing to follow the Consensus Statement for Standard of Care in Spinal Muscular Atrophy.
7. Parent(s)/legal guardian(s) willing and able to complete the informed consent process and comply with study procedures and visit schedule
8. Genetic diagnosis as described below, obtained from an acceptable newborn or pre-natal screening test method

Patients with 2 copies of *SMN2* (n ≥14)

- Patients with pre-symptomatic SMA Type 1 as determined by the following features:
  - 2 copies of *SMN2*

Patients with 3 copies of *SMN2* (n ≥12)

- Patients with pre-symptomatic SMA Type 2 as determined by the following features:
  - 3 copies of *SMN2*

Exclusion Criteria:

1. Weight at screening visit <2 kg
2. Hypoxemia (oxygen saturation <96% awake or asleep without any supplemental oxygen or respiratory support) at the screening visit or for altitudes >1000 m, oxygen saturation <92% awake or asleep without any supplemental oxygen or respiratory support at the screening visit
3. Any clinical signs or symptoms at screening or immediately prior to dosing that are, in the opinion of the Investigator, strongly suggestive of SMA (e.g., tongue fasciculation, hypotonia, areflexia)
4. Tracheostomy or current prophylactic use or requirement of non-invasive ventilatory support at any time and for any duration prior to screening or during the screening period
5. Patients with signs of aspiration/inability to tolerate non-thickened liquids based on a formal swallowing test performed as part of screening or patients receiving any non-oral feeding method
6. Clinically significant abnormal laboratory values (GGT, ALT, and AST, or total bilirubin > 2 × the 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.
7. Patients with any other clinically significant abnormalities in hematology or clinical chemistry parameters as determined by the investigator or medical monitor
8. Treatment with an investigational or commercial product, including nusinersen, given for the treatment of SMA. This includes any history of gene therapy, prior antisense oligonucleotide treatment, or cell transplantation.
9. Patients whose weight-for-age is below the third percentile based on World Health Organization (WHO) Child Growth Standards.
10. Biological mother with active viral infection as determined by screening laboratory samples (includes human immunodeficiency virus [HIV] or positive serology for hepatitis B or C)
    a. Biological mothers with clinical suspicion of Zika virus that meet Centers for Disease Control and Prevention (CDC) Zika virus epidemiological criteria including history of residence in or travel to a geographic region with active Zika transmission at the time of travel will be tested for Zika virus ribonucleic acid (RNA); positive results warrant confirmed negative Zika virus RNA testing in the patient prior to enrollment
11. Serious non-respiratory tract illness requiring systemic treatment and/or hospitalization within 2 Weeks prior to screening
12. Upper or lower respiratory infection requiring medical attention, medical intervention, or increase in supportive care of any manner within 4 Weeks prior to dosing
13. Severe non-pulmonary/respiratory tract infection (e.g., pyelonephritis, or meningitis) within 4 Weeks before administration of gene replacement therapy or concomitant illness that, in the opinion of the Investigator or Sponsor medical monitor, creates unnecessary risks for gene replacement therapy such as:
    a. Major renal or hepatic impairment
    b. Known seizure disorder
    c. Diabetes mellitus
    d. Idiopathic hypocalciuria
    e. Symptomatic cardiomyopathy

14. Known allergy or hypersensitivity to prednisolone or other glucocorticosteroids or their excipients
15. Previous, planned or expected major surgical procedure including scoliosis repair surgery/procedure during the study assessment period
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, immunosuppressive therapy within 4 Weeks prior to gene replacement therapy (e.g., corticosteroids, cyclosporine, tacrolimus, methotrexate, cyclophosphamide, IV immunoglobulin, rituximab)
17. Anti-AAV9 antibody titer >1:50 as determined by enzyme-linked immunosorbent assay (ELISA) binding immunoassay
    a. Should a potential patient demonstrate Anti-AAV9 antibody titer >1:50, he or she may receive retesting inside the 30-Day screening period and will be eligible to participate if the Anti-AAV9 antibody titer upon retesting is ≤1:50, provided patient is still <6 weeks of age at the time of dosing
18. Biological mother involved with the care of the child refuses anti-AAV9 antibody testing prior to dosing
19. Parent(s)/legal guardian(s) unable or unwilling to comply with study procedures or inability to travel for repeat visits
20. Parent(s)/legal guardian(s) unwilling to keep study results/observations confidential or to refrain from posting confidential study results/observations on social media sites
21. Parent(s)/legal guardian(s) refuses to sign consent form

Investigational Product, Dosage and Mode of Administration:

Patients will receive a one-time dose of AVXS-101 at 1.1×10^{14} vg/kg.

Duration of Treatment:

AVXS-101 will be administered as a one-time IV infusion over approximately 60 minutes

Reference Therapy, Dosage and Mode of Administration: Not Applicable

Definition for Endpoints (alphabetically):

Chronological development reference standard: Fine and Gross motor development will reference and be assessed using the BSIDv03 Gross and Fine Motor Subsets

Functional independent sitting: Sitting without support [redacted] (BSIDv03 – Gross Motor subset #26)

Permanent Ventilation: Tracheostomy or the requirement of ≥16 hours of respiratory assistance per Day (via non-invasive ventilatory support) for ≥14 consecutive days in the absence of an acute reversible illness, excluding perioperative ventilation. Permanent ventilation, so defined, is considered a surrogate for death.

Respiratory Intervention: Use of ventilatory support (Bi-level Positive Airway Pressure (BiPAP) or other ventilator) for ≥14 consecutive days in the absence of an acute reversible illness, excluding perioperative ventilation

Standard deviation: 7+ points on the scaled scoring for the gross and fine motor subsets of the BSIDv03.

Standing alone: Stand without support for at least 3 seconds (BSIDv03 Gross Motor Subset #40)

Survival: Avoidance of death or the requirement of permanent ventilation (including non-invasive ventilatory support ≥ 16 hours per Day for ≥ 2 weeks) in the absence of acute reversible illness or perioperatively

Walking alone: [redacted] (BSIDv03 – Gross Motor Subset #43)

Criteria for Evaluation:

Safety:

Primary:

- Incidence of AEs and/or SAEs
- Change from baseline in clinical laboratory parameters

Efficacy objectives will be assessed independently for each cohort.

Efficacy for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

Primary:

- Proportion of patients achieving the development milestone of functional independent sitting as defined by BSIDv03 Gross Motor Subtest Item #26 at any visit up to 18 months of age

Secondary:

- Proportion of patients that have survived and have not required permanent ventilation in the absence of acute reversible illness or perioperatively assessed at 14 months of age
- Proportion of patients that have achieved the ability to maintain weight at or above the third percentile without need for non-oral/mechanical feeding support at any visit up to 18 months of age

Exploratory:

[redacted]

[redacted]

Efficacy for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

Primary:

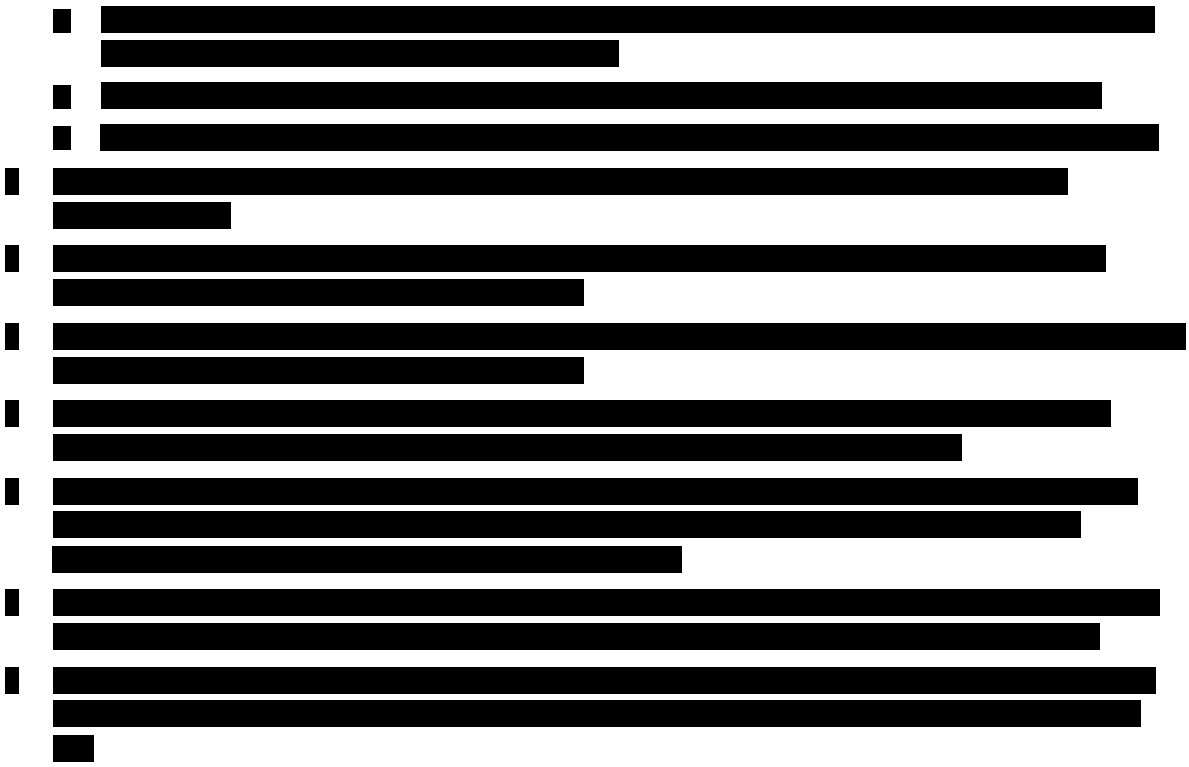
- Proportion of patients achieving the ability to stand without support for at least 3 seconds as defined by BSIDv03 Gross Motor Subtest Item #40 at any visit up to 24 months of age

Secondary:

- Proportion of patients demonstrating the ability to walk alone[redacted] as defined by BSIDv03 Gross Motor Subtest Item #43 at any visit up to 24 months of age

Exploratory:

[redacted]



Statistical Methods:

This is a Phase 3 study assessing the efficacy and safety of AVXS-101. The primary efficacy analysis for each *SMN2* copy number cohort will be completed separately at such time that enrollment in the respective cohort is complete and the last patient has completed the EOS visit at the respective age (*SMN2* = 2, EOS = 18 months of age; *SMN2* = 3, EOS = 24 months of age). Safety monitoring will continue for 30 days after the EOS visit. Details of all analyses will be contained within the Statistical Analysis Plan.

The primary and secondary efficacy analyses will be performed on the populations of patients with bi-allelic *SMN1* deletions and 2 or 3 copies of *SMN2* without the *SMN2* gene modifier mutation (c.859G>C). While the study may enroll a small number of additional patients, the above will comprise the population for evaluation of the primary and secondary endpoints and will be referred to as the Intent-To-Treat (ITT) populations hereafter. Study power is based upon efficacy analysis of these subpopulations. Patients with *SMN1* point mutations or the *SMN2* gene modifier mutation (c.859G>C) will be evaluated separately as part of additional subgroup analyses.

At least fourteen (14) patients with 2 copies of *SMN2* and at least twelve (12) patients with 3 copies of *SMN2* will be enrolled.

Patients with 2 copies of *SMN2*

The primary efficacy endpoint for patients with 2 copies of *SMN2* will be the proportion of patients who achieve the ability to sit without support [redacted] at any visit up to the 18 months of age study visit. Based upon widely cited natural history of the disease (Pediatric Neuromuscular Clinical Research Network [PNCR]), it is expected that no patient meeting the ITT criteria (bi-allelic *SMN1* deletion, *SMN2* copy number of 2 without the *SMN2* gene modifier mutation (c.859G>C)) would be expected to attain the ability to sit without support. Based upon data from the completed AVXS-101-CL-101 study, we expect at least 60% of treated patients with 2 copies of *SMN2* to achieve the ability to sit without support for at least 30 seconds. With this efficacy, a sample size of at least 14 patients who meet the cohort ITT criteria would provide power of >90% to detect a significant

difference compared with a rate of 0.1% (in lieu of zero) with $\alpha = 0.025$ using a 1-sided exact test for a binomial proportion.

Patients with 3 copies of *SMN2*

The primary efficacy endpoint for patients with 3 copies of *SMN2* will be the proportion of patients who achieve the ability to stand without support for at least 3 seconds at any visit up to the 24 months of age study visit. Based upon patient-level data available from the PNCR dataset, 23.5% of patients with *SMN2*=3 who meet the ITT criteria (bi-allelic *SMN1* deletions, 3 copies of *SMN2*, absence of *SMN2* gene modifier mutation (c.859G>C)) achieved the ability to stand without support. We expect 85% of treated patients with 3 copies of *SMN2* to achieve the ability to stand without support. With this efficacy, a sample size of at least 12 patients that meet the ITT criteria for this cohort would provide power of >90% to detect a significant difference compared with the matched control cohort with $\alpha = 0.05$ using a 2-sample 2-sided superiority Fisher exact test.

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

LIST OF TABLES

LIST OF FIGURES

4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

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

Abbreviation or Specialist Term	Explanation
AAV	Adeno-associated virus
AAV9	Adeno-associated virus serotype 9
ADL	Activities of daily living
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AT	Aminotransferase
BSID	Bayley Scales of Infant and Toddler Development
BUN	Blood urea nitrogen
CB	Chicken-β-actin-hybrid
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CHOP INTEND	Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
Chronological development reference standard	Fine and Gross motor development will reference and be assessed using the Bayley Scales of Infant and Toddler Development© V.3 Gross and Fine Motor Subsets
CK	Creatine kinase
CK-MB	Creatine kinase isoenzyme
CMAP	Compound motor action potential
CMV	Cytomegalovirus
CNS	Central nervous system
CTCAE	Common Terminology Criteria for Adverse Events
Day 1	First 24-hour interval after the start of gene replacement therapy infusion
Day –1	24-hour interval prior to the start of gene replacement therapy infusion
ddPCR	Droplet digital polymerase chain reaction
DMC	Data Monitoring Committee
DRG	Dorsal root ganglia
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
ELISpot	Enzyme-linked ImmunoSpot
EOS	End of Study
ET	Early Termination

Abbreviation or Specialist Term	Explanation
Functional independent sitting	Sitting without support for ≥30 seconds (Bayley Scales of Infant and Toddler Development v.3 – Gross Motor subset #26)
FVB	Friend Virus B-Type
GCP	Good Clinical Practice
GFP	Green fluorescent protein
GGT	Gamma glutamyl transferase
GLP	Good Laboratory Practice
HEENT	Head, eyes, ears, nose, and throat
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
IMP	Investigational medicinal product
IRB	Institutional Review Board
ITR	Inverted terminal repeat
ITT	Intent-to-treat
IV	Intravenous
LFT	Liver function test
NHP	Non-human primate
MedDRA	Medical Dictionary for Regulatory Activities
NHP	Non-human primates
NOAEL	No Observable Adverse Effect Level
OAE	Other Adverse Event
PBMC	Peripheral blood mononuclear cells
Permanent Ventilation	Tracheostomy or the requirement of ≥16 hours of respiratory assistance per Day (via non-invasive ventilatory support) for ≥14 consecutive days in the absence of an acute reversible illness, excluding perioperative ventilation. Permanent ventilation, so defined, is considered a surrogate for death.
PICU	Pediatric intensive care unit
PNCR	Pediatric Neuromuscular Clinical Research Network
rAAV	Recombinant Adeno-associated virus
Respiratory intervention	Use of ventilatory support (Bi-level Positive Airway Pressure (BiPAP) or other ventilator) for ≥14 consecutive days in the absence of an acute reversible illness, excluding perioperative ventilation
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
SAE	Serious adverse event
SAP	Statistical Analysis Plan
scAAV	Self-complimentary adeno-associated virus

Abbreviation or Specialist Term	Explanation
scAAV9.CB.SMN	Self-complimentary adeno-associated virus serotype 9 chicken-β-actin-hybrid survival motor neuron
SMA	Spinal muscular atrophy
SMN	Survival motor neuron
SMN1	Survival motor neuron 1 gene
SMN2	Survival motor neuron 2 gene
SoA	Schedule of Assessments
Standard deviation	7+ points on the scaled scoring for the gross and fine motor subsets of the Bayley Scales of Infant and Toddler Development© V.3
Standing alone	Stand without support for at least 3 seconds (Bayley© Scales of Infant and Toddler Development – Gross Motor Subset #40)
Survival	Avoidance death or the requirement of permanent ventilation (including non-invasive ventilatory support ≥16 hours per Day for ≥2 weeks) in the absence of acute reversible illness or perioperatively
SUSAR	Suspected unexpected serious adverse reaction
TBL	Total bilirubin
ULN	Upper limit normal
US	United States
vg/kg	Vector genome per kilogram
Walking alone	[redacted] (Bayley© Scales of Infant and Toddler Development – Gross Motor Subset #43)
WBC	White blood cell
WHO	World Health Organization
WHO-MGRS	World Health Organization Multcentre Growth Reference Milestones
wtAAV	Wild type Adeno-associated virus
WT	Wild type

5. INTRODUCTION

Study AVXS-101-CL-304 is a Phase 3 clinical gene therapy study investigating the efficacy and safety of a single intravenous (IV) infusion of AVXS-101 in up to 26 patients with spinal muscular atrophy (SMA) having either 2 or 3 copies of survival motor neuron 2 gene (*SMN2*). 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 SMNΔ7 mouse model for SMA from a median of 15.5 days to over 1 year, following IV delivery to a facial vein. Additionally, results from a completed Phase 1 clinical study (AVXS-101-CL-101) of AVXS-101 in SMA Type 1 patients demonstrates broad improvements in motor function, pulmonary function, and nutritional function (Section 5.4) as well as survival beyond 20 months of age for 100% of treated patients.

5.1. Background

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. Spinal muscular atrophy is an autosomal recessive, early childhood disease with an incidence of approximately 1: 10,000 live births (1). Spinal muscular atrophy 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 before 6 months of age and early death due to respiratory failure before 2 years of age. Motor neuron loss in SMA Type 1 is profound in the early post-natal period (or may even start in the prenatal period), whereas motor neuron loss in SMA Type 2 and Type 3 patients is less profound and a greater population of neurons is able to survive 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 post-natal periods (2-4). From a clinical perspective, these findings emphasize the importance of targeting pre-symptomatic SMA patients, regardless of their ultimate disease course, for gene transfer of *SMN1* in hopes of rescuing neurons at this critical stage. The goal in continuing the development plan for AVXS-101 is to modify the SMA phenotype, which will hopefully lead to a milder disease course, reduced morbidity and prolonged survival for all patients with SMA.

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, phenylbutyrate, and trichostatin A. These agents activate the *SMN2* promoter, resulting in increased full-length SMN protein in SMA animal models (5, 6); however, clinical studies employing several of these agents, most notably phenylbutyrate, valproic acid, and hydroxyurea, have not resulted in clinical benefit (7, 8). 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 intrathecal injection, requires a lengthy induction period prior to effectiveness, and has safety considerations which require clinical monitoring. A single-dose IV administration study of AVXS-101 in pre-symptomatic patients

will provide information on the potential gene transfer has in treating SMA patients early and will hopefully show promise for success in modifying the disease prognosis.

This is a single-dose study that will include up to 26 patients with 2 or 3 copies of *SMN2*. The rationale for IV dosing is based upon the need for rapid, systemic impact given the severity of the disease in SMA and its potential impact on systems outside of the central nervous system (CNS) such as the peripheral and autonomic nervous systems, heart, pancreas and gastrointestinal tract.

5.2. Rationale for Gene Transfer to Pre-Symptomatic SMA Patients

Patients with pre-symptomatic genetically diagnosed SMA have been chosen as the target population for this gene therapy study-based on studies of the natural history of this disease. The classification of SMA is shown below (Table 1) in which SMA Types 0 to 4 are described. Spinal muscular atrophy is conventionally classified into 4 phenotypes on the basis of age at onset and highest motor function achieved, with an additional phenotype (Type 0) to describe the severe forms of antenatal-onset SMA.

As discussed in Section 5.4: Clinical Studies, a robust clinical response was seen amongst symptomatic patients with 2 copies of *SMN2* and early symptom onset consistent with SMA Type 1, with 9 of 12 patients treated at the proposed therapeutic dose achieving the ability to sit independently, and 11 of 12 surpassing a Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) score of 40 points, a threshold not achieved by any untreated type 1 patients older than 6 months of age. There appears to be a relationship between baseline age and function and motor function achievement. One patient treated at almost 8 months of age with poor baseline function showed modest response to treatment; in contrast two patients treated within the first 2 months of life with relatively modest symptoms have showed a dramatic clinical response and are both walking independently. This suggests that there is a critical window to treat before significant motor neuron loss has occurred to achieve the optimal response to intervention with AVXS-101. Under this paradigm, treating patients in a pre-symptomatic setting represents the idealized approach to therapeutic intervention in this disease.

Table 1 - 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	4-8

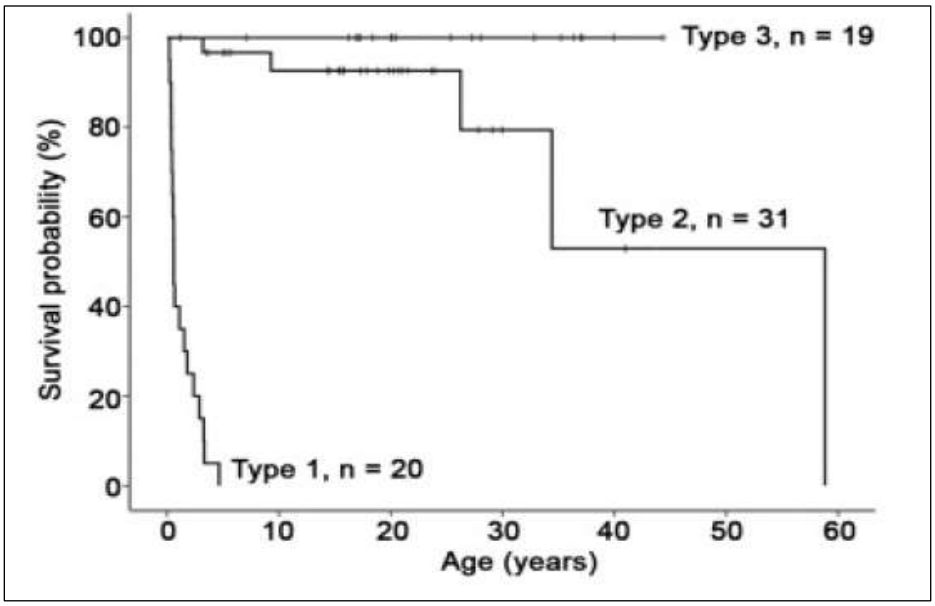
Source: Adapted from Kolb 2011 (9)
SMN2 = survival motor neuron 2 gene
bold = predominant SMN2 copy number that defines the SMA Type, the other copy numbers represent a small percentage of the designated SMA Type.

Spinal muscular atrophy Type 1 patients, by definition, never attain independent sitting and have hypotonia within the first 6 months of life. Spinal muscular atrophy Type 1 is the leading genetic cause of infant death with an onset at ≤6 months of age (Table 1). 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. Spinal muscular atrophy Type 3 patients attain the ability to walk unaided (Type 3a have onset before 3 years of age; Type 3b have onset >3 years of age). Spinal muscular atrophy 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* gene.

Humans also carry a second nearly identical copy of the *SMN1* gene called *SMN2* (10). Both the *SMN1* and *SMN2* genes express SMN protein; however, the amount of functional full-length protein produced by *SMN2* is only 10 to 15% of that produced by *SMN1* (10-12). Although *SMN2* cannot completely compensate for the loss of the *SMN1* gene, patients with milder forms of SMA generally have higher *SMN2* copy numbers (13, 14). Quantitative analysis of *SMN2* copies in 375 patients with Type 1, 2, or 3 SMA showed a significant correlation between *SMN2* copy number and SMA Type, as well as, duration of survival. In a large early study by Feldkotter et al 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 (15). As these percentages do not reflect the possible impact of modifier mutations such as that described by Prior et al 2009 (16), they may understate the relationship between copy number (in the absence of a genetic modifier) and clinical phenotype. 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 to 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 1 is demonstrated by prognosis as illustrated in Kaplan-Meier survival curves shown in .

Figure 1 - Kaplan-Meier Survival Curves and Survival Probabilities for SMA Types 1, 2, and 3



n = number of patients
Source: Farrar 2013 (17)

In , the relative stability of the clinical course of SMA Type 2 and Type 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 post-natal period (or may even start in the prenatal period, especially for SMA Type 1 patients presenting in first 3 months of life). The findings in confirm other pieces of evidence from neurophysiological studies and animal studies that also show early loss of motor neurons in the embryonic and early post-natal periods (2-4).

It is important to note that the classification system used in SMA is based upon best function (and expectation of best function extrapolated from clinician experience at the time of diagnosis) in a clinical landscape devoid of disease modifying therapies. Given the close relationship between *SMN2* copy number and expected clinical course, it makes more sense to approach the disease in a prospective manner from the perspective of *SMN2* copy number rather than clinical symptoms that will no longer be fully manifested in effectively treated patients.

There is reason to believe that there are few safety issues to be concerned about when targeting pre-symptomatic, genetically diagnosed SMA patients in this gene therapy clinical study. Overexpression of SMN has been shown to be well tolerated in both mice and non-human primates (NHPs), and in humans, a high copy number of *SMN2* poses no risk (as seen in Type 2, 3, and 4 patients who have high *SMN2* copy number), allowing for use of 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 study 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.

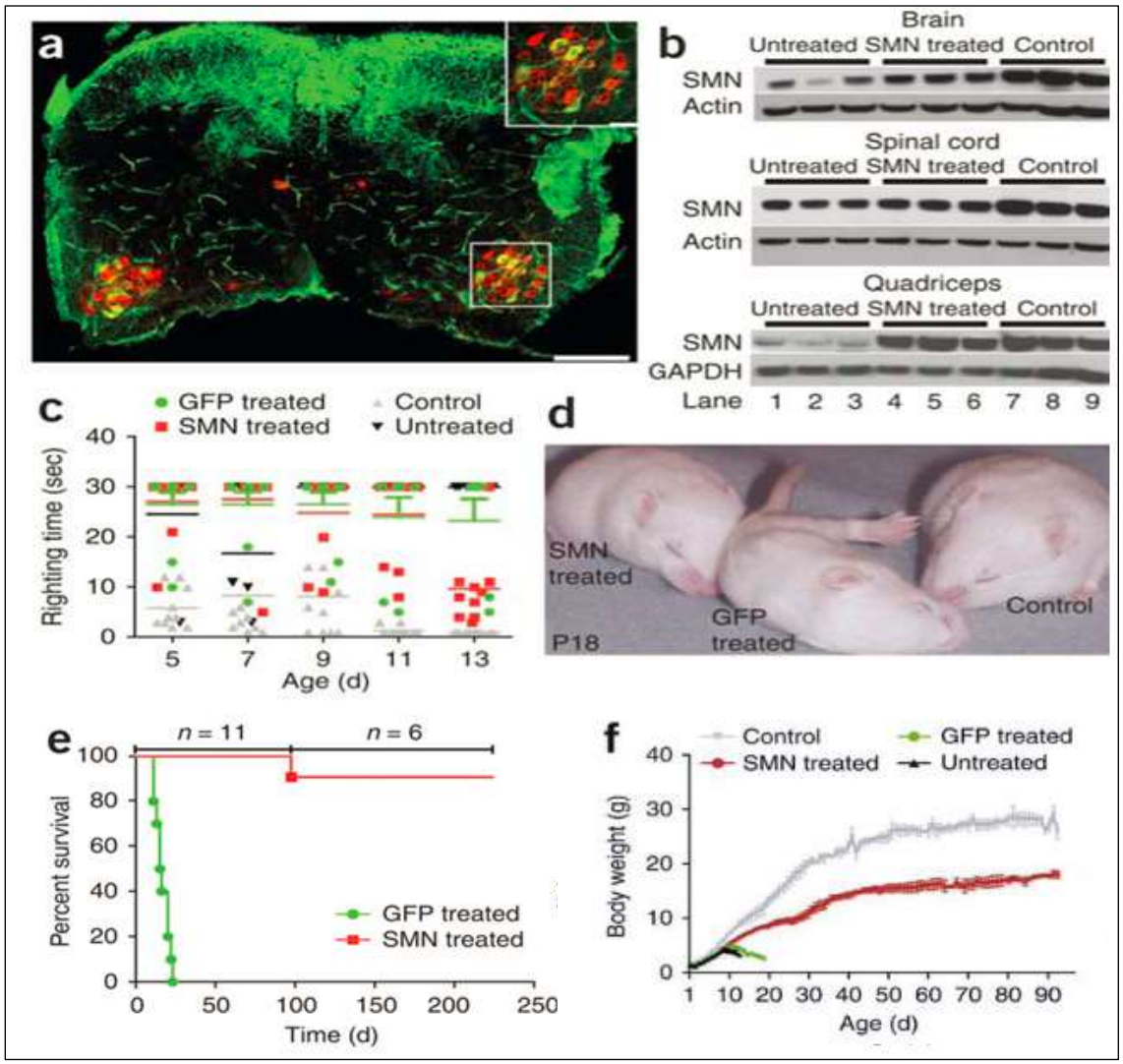
Recent studies using self-complimentary adeno-associated virus serotype 9 chicken-β-actin-hybrid survival motor neuron (scAAV9.CB.SMN) show a robust post-natal rescue of SMNΔ7 mice with correction of motor function, neuromuscular electrophysiology and survival after a one-time delivery of the vector (18). Intravenous scAAV9 is able to transduce neurons, muscle and vascular endothelium, all of which have been proposed as target cells for SMA treatment.

5.3. Nonclinical Studies

A mouse model was developed by the [redacted] after a generation of multiple variants. It was found that the double transgenic, referred to as the SMNΔ7 mouse, provided the most suitable model to study gene transfer (19). Studies performed in the [redacted] have shown that injecting 5×10^{11} viral genomes of scAAV9.CB.SMN into the facial vein on Day 1 old mice rescues the SMNΔ7 mouse model (18). 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.GFP treated mice. SMN transduction was shown by real time polymerase chain reaction (RT-PCR) in the mice. Green fluorescent protein (GFP) transduction was observed by microscopy. Both constructs were in adeno-associated virus serotype 9 (AAV9) and had transduction of motor neurons. SMN levels were increased as well, in brain, spinal cord, and muscle of scAAV9.CB.SMN-treated animals, compared to untreated SMNΔ7 mice (although lower than wild type [WT] controls). SMNΔ7 animals treated with either scAAV9.CB.SMN or scAAV9.CB.GFP on post-natal Day 1 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 SMNΔ7 animals compared with GFP-treated SMNΔ7 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

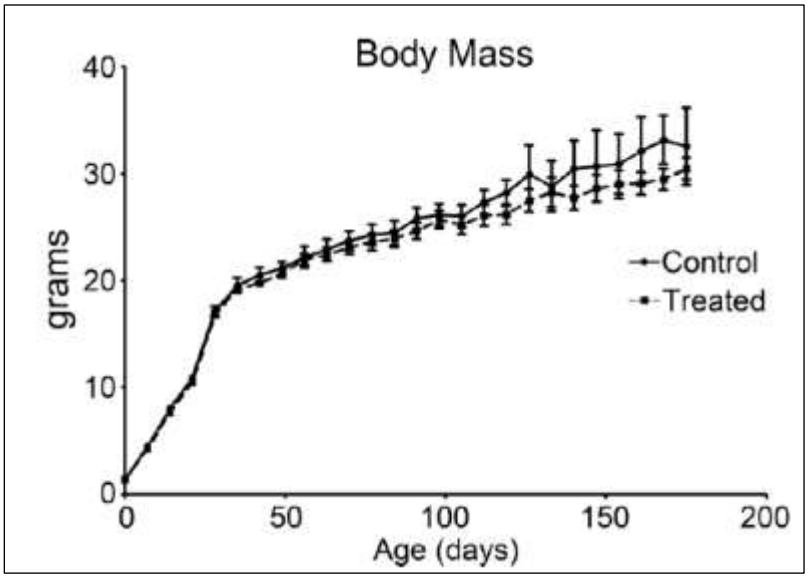


Source: Foust 2010 (18)
CNS = central nervous system; GFP = green fluorescent protein; SMN = survival motor neuron; WT = wild type
a) Shows transduced motor neurons in lumbar spinal cord
b) Western Blots of SMN expression in CNS and muscle
c) Improved righting ability of SMN-treated- similar to WT controls 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 [redacted] [data on file]. In these non-Good Laboratory Practice (GLP) studies, 24 mice and 4 NHPs were injected, by way of vascular delivery, with AVXS-101. To assess toxicity and safety, AVXS-101 was injected into P1 WT Friend Virus B-Type (FVB) mice with either vehicle (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 SMNΔ7 mouse model (18). P1 mice were used in anticipation of simulating potential clinical studies in infants. 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 - 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 including complete blood counts with differentials. At 90, 120 and 180 days post-injection, blood was collected for clinical chemistries assessment (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, creatinine, blood urea nitrogen [BUN], electrolytes, and creatine kinase [CK]). For histopathology, 13 mice were necropsied at 120 days post-injection and 8 mice at 180 days. There were no clinically significant results observed during from the hematology, clinical chemistry, and histopathology portions of the study and trends of both groups were comparable. Of note, no significant lesions were present in any brain or spinal cord sections, although, the sections were frozen and thicker than 5 microns which made cellular morphology obscure and subtle changes may not have been identified.

In the safety study for the 4 male cynomolgus macaques, animals were injected at 90 days of age to closely mimic the likely age of administration of treatment in SMA Type 1 infants. The AVXS-101 vector was administered one time by catheterization of the saphenous vein with a dose of 6.7×10^{13} vg/kg, which corresponds to the lowest dose tested for which SMNΔ7 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, the serum chemistry and hematology data were unremarkable as was the histopathology assessment. The NHPs 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 [data on file].

When newborn FVB mice were given a single IV injection of AVXS-101 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 timepoints 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 (APTT) values, were mild effects of treatment, but did not result in toxicity. Activity of AVXS-101 was demonstrated by the biodistribution 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.

As part of the preclinical development of AVXS-101 for intrathecal delivery, an exploratory non-GLP biodistribution and safety study was performed in cynomolgus monkeys (*Macaca fascicularis*) to evaluate the transduction efficiency and safety of intrathecally administered AVXS-101 at a dose of 3×10^{13} vg/animal alone and in combination with 2 iohexol-based contrast agents. All 12 animals on study survived and were euthanized 2 weeks post-injection with no clinical evidence of toxicity. However, inflammation of the dorsal root ganglia (DRG) was noted during histopathology evaluation of select tissues. The inflammation was characterized by minimal to marked infiltration of mononuclear inflammatory cells, primarily lymphocytes, into the cervical, thoracic, lumbar, and sacral DRGs and associated nerves. Minimal inflammation was associated with scattered infiltrates or small aggregates of mononuclear cells in the DRG, without evidence of neuronal necrosis. With mild to marked inflammation, aggregates to sheets of mononuclear cells were present, along with neuronal satellitosis, neuronal necrosis, or neuronal loss with rare mineralization. Inflammation was observed in ganglia from all examined levels, but incidence and severity were generally greater in the sacral DRG. Moderate to marked inflammation was only observed in the sacral DRG of two of the twelve animals on study. The animals were not administered corticosteroids.

The DRG was not identified as a target organ of toxicity in previous AVXS-101 studies conducted in mice (ICV route of administration) or cynomolgus monkeys (IV or IT routes of administration). However, similar findings have been reported after administration of AAV9 vectors in monkeys and minipigs (20, 21).

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 event 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.

5.4. Clinical Studies

First-in-human study AVXS-101-CL-101 is a completed 2-year study 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: Cohort 1 (n = 3) received 6.7×10^{13} vg/kg and Cohort 2 (n = 12) received 2.0×10^{14} vg/kg (proposed therapeutic dose). 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 at all 3 time points 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 (22).
- Based on independent video reviews, developmental milestones were achieved and maintained over time.
    - In Cohort 2, 11 patients (91.7%) could hold their head erect without support for ≥ 3 seconds and sit with support, 9 patients (75.0%) were able to sit without support for ≥ 30 seconds, and 2 patients each (16.7% each) were able to stand with assistance, stand alone, walk with assistance, and walk alone
- The improvements in CHOP INTEND scores from baseline in both cohorts were sustained over time.
- Nearly all Cohort 2 patients (11/12, 91.7%) achieved a score ≥ 50 on the CHOP INTEND, approximating the range of scores for Type 2 SMA children reported in the Pediatric Neuromuscular Clinical Research Network (PNCR) study (22).
- Other clinically meaningful developmental milestones were maintained 24 months after AVXS-101 infusion:
    - Of the 7 patients in Cohort 2 who did not require non-oral nutrition prior to AVXS-101 dosing, 5 (71.4%) maintained the ability to thrive
    - Eleven Cohort 2 patients (91.7%) were still able to swallow effectively enough to feed orally
    - Seven of 15 patients (46.7%) remained independent of ventilatory support

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 (23).

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 (24). The relevance to humans with SMA is unknown.

Respiratory tract infections in neonates are very common in the general pediatric population (25). 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 (26). RSV is the primary cause of hospitalization for respiratory tract infection in young children (27). 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 (28), are also recommended (29) 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 (Section 14.4.1), did not exhibit clinically relevant increases in bilirubin, and generally resolved with prednisolone treatment. A case of acute liver failure (suspected unexpected serious adverse reaction [SUSAR]) was reported in the United States (US) Managed Access Program with AVXS-101. A 6-month-old male concurrently receiving nusinersen with elevations of AST and ALT of > 3 x 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 IB, there have been no cardiovascular AEs that have been judged to be related to AVXS-101 in the clinical studies. There have been no persistent clinically significant electrocardiogram (ECG) changes. In Study AVXS-101-CL-303, a Phase 3 open-label single-dose study in patients with one or two copies of SMN2, elevated CK-MB was reported for one patient, but this TEAE was determined by the Investigator to be unrelated to AVXS-101 administration. There have been no AEs reported for troponin-I. In Study 101, there were minor transient increases in troponin-I at the proposed therapeutic dose in Cohort 2 with no associated clinical sequelae. None of the elevations in cardiac troponin-I observed during the study were considered clinically significant by the investigator. Small mean troponin elevations were minimally apparent during the first two months post-dose. Other troponin elevations appeared to be sporadic. By the end of the study all values had either returned to within the normal range or no longer met the pre-defined criterion for potential clinical significance. The autopsy report from Study 303 patient, whose death was unrelated to AVXS-101, indicates that the heart was macroscopically and microscopically normal, with sections of the heart having no pathologic diagnosis. While the clinical significance of the transiently elevated troponin-I levels is unknown, it is recommended that troponin-I be monitored for the first 3 months following dosing.

A transient decrease in platelet counts has been observed in Studies 101, 303, and 102 typically at Day 7. The majority of values remained above the lower limit of normal. Decreases were clinically asymptomatic and transient.

Preclinical data indicate that in most cases, DNA delivered by recombinant Adeno-associated virus (rAAV) vectors predominantly persists as extrachromosomal elements (episomes) rather than integrating into host cell genomes (30). Although AVXS-101 is also not anticipated to integrate into the host cell genome as described above, the long-term consequences of administering AAV viral vectors to humans are not yet fully understood. This is in contrast to wild type adeno-associated virus (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 (31, 32). Since the AVXS-101 product uses AAV9 with all of the WT DNA 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 wild-type AAV2 genome is associated with induction of hepatocellular carcinoma in a small subset of patients; however, there are several studies with evidence to contradict these claims including: a) AAV2 has infected approximately 90% of the human population, b) AAV2 has been shown to possess anticancer activity, c) epidemiological evidence suggests that AAV2 infection plays a protective role against cervical carcinoma, and d) AAV serotypes including recombinant AAV2 and AAV9 have been or are currently used in 162 clinical trials to date in which no cancer of any type has been observed or

reported. For a review of the topic, see Srivastava and Carter, 2017 (33). 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 NHPs exposed to AVXS-101.

It is possible the AAV9 vector containing the SMN gene could interact with other viruses with which the patients come in contact, such as rhinoviruses, adenovirus, or herpes. If this happens, the AAV9 vector could form a virus that causes infection if the patient and cells for rescue, replication, and packaging are also exposed to wild-type AAV2. The rescue, replication, and packaging would stop; however, as the helper viruses, such as rhinoviruses, adenovirus, or herpes were cleared by the patient's immune system. This unlikely scenario has been studied. In cell culture, the rAAV genome can be rescued and replicated by superinfection with wtAAV and a helper virus; however, in vivo rescue experiments have failed to show rescue and replication (34), except in one case in which very large doses of wtAAV and adenovirus were administered in a particular setting. 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.

AVXS-101 is detectable in the shed samples from Day 1 post injection. Clearance of AVXS-101 is primarily via the feces and the majority of the dose is cleared within 30 days of dose administration. While initially concentrated in stool, the amount of vector shed declines logarithmically. In stool, levels 10% to 30% of the initial concentration in the body were detectable at Day 1 post infusion. At Day 14 post-infusion one patient showed a peak concentration in stool of 280% of initial concentration in body. In contrast, 3 patients for whom data were available showed a concentration of <1% of initial concentration in body at Day 14 post infusion. These concentrations declined approximately 4 logs over 30 days post dose, and all patients had levels of AVXS-101 in stool below the ddPCR limit of quantitation by 60 days post dose. Concentrations of vector shed in saliva and urine are quite low and are below the ddPCR limits of quantitation in the matrices within days post-dose. AVXS-101 concentrations representing 0.1% to 0.01% of the initial dose (concentration) into the patient are found in urine and saliva at Day 1 post infusion, after which concentrations fell below the limit of quantitation

of the assay [data on file]. Shed AAV vectors have been previously shown not to be infectious in urine and saliva excreta (34). Together, these data demonstrate rapid decline of shed vector quantities well below initial concentrations in patients treated with AVXS-101.

In an exploratory non-GLP study, inflammation of the DRG (characterized by infiltration of mononuclear inflammatory cells, along with neuronal satellitosis and neuronal necrosis or loss) was observed 14 days after cynomolgus monkeys were administered AVXS-101 intrathecally. Although the inflammation was observed in ganglia from all examined levels (cervical, thoracic, lumbar, and sacral), the incidence and severity were generally greater in the sacral DRG. The clinical relevance of the DRG findings in cynomolgus monkeys is unknown at this time. No steroids were administered in the animal study (Section 5.3).

A thorough review of the safety data from patients treated with AVXS-101, based on a data cutoff of 31 December 2019, did not identify any AEs related to sensory changes. Nevertheless, study participants will be closely monitored for any clinical manifestations of a potential DRG involvement.

Finally, there are potential risks associated with IV dosing. There may be a pain at the site of the infusion as well as bruising surrounding the infusion site. Infections are also possible at the site of the infusion.

These results also strongly support further clinical investigation of the efficacy and safety of AVXS-101 in patients with pre-symptomatic SMA. Because SMA is a progressive neurodegenerative disease which involves the irreversible neurodegeneration and death of motor neurons beginning before the onset of clinical symptoms, administration of gene replacement therapy as early as possible is believed to be of substantial importance for preventing motor neuron loss, maximizing the opportunity for patients to experience meaningful benefits from this treatment. This hypothesis is supported by data from the AVXS-101-CL-101 clinical study, which has included observations of substantial benefits in survival, motor function, and developmental milestone achievements relative to natural history, which were particularly striking for several patients treated at younger ages. Based on the current available data, safety events that appear to be associated with AVXS-101 consist of transient liver enzyme elevations, and which have resolved following treatment with prednisolone. Considering this favorable developing safety profile together with the potential for highly meaningful clinical benefits of early treatment, the risk-benefit relationship strongly supports further clinical investigation of AVXS-101 in infants with pre-symptomatic SMA.

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

6. STUDY OBJECTIVES AND PURPOSE

6.1. Safety Objectives

6.1.1. Primary Objectives

The safety objectives are to:

- Evaluate the safety of AVXS-101 through incidence of AEs and/or serious adverse events (SAEs)
- Assess the safety of AVXS-101 based on the change from baseline in clinical laboratory parameters

6.2. Efficacy Objectives

Efficacy objectives will be assessed independently for each cohort.

6.2.1. Patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*

6.2.1.1. Primary Objective (2 copy)

Assess the efficacy of AVXS-101 by demonstrating functional independent sitting for [redacted] as defined by Bayley Scales of Infant and Toddler Development© Version 3 (BSIDv03) Gross Motor Subtest Item #26 at any visit up to 18 months of age.

6.2.1.2. Secondary Objective (2 copy)

Secondary objectives include:

- Assess the efficacy of AVXS-101 based on survival, defined as avoidance of death or the requirement of permanent ventilation in the absence of acute illness or perioperatively as assessed at 14 months of age
- Assess efficacy of AVXS-101 by demonstrating the ability to maintain weight at or above the third percentile without need for non-oral/mechanical feeding support at any visit up to 18 months of age

6.2.1.3. Exploratory Objectives (2 copy)

[redacted]

[redacted]

6.2.2. Patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*

6.2.2.1. Primary Objectives (3 copy)

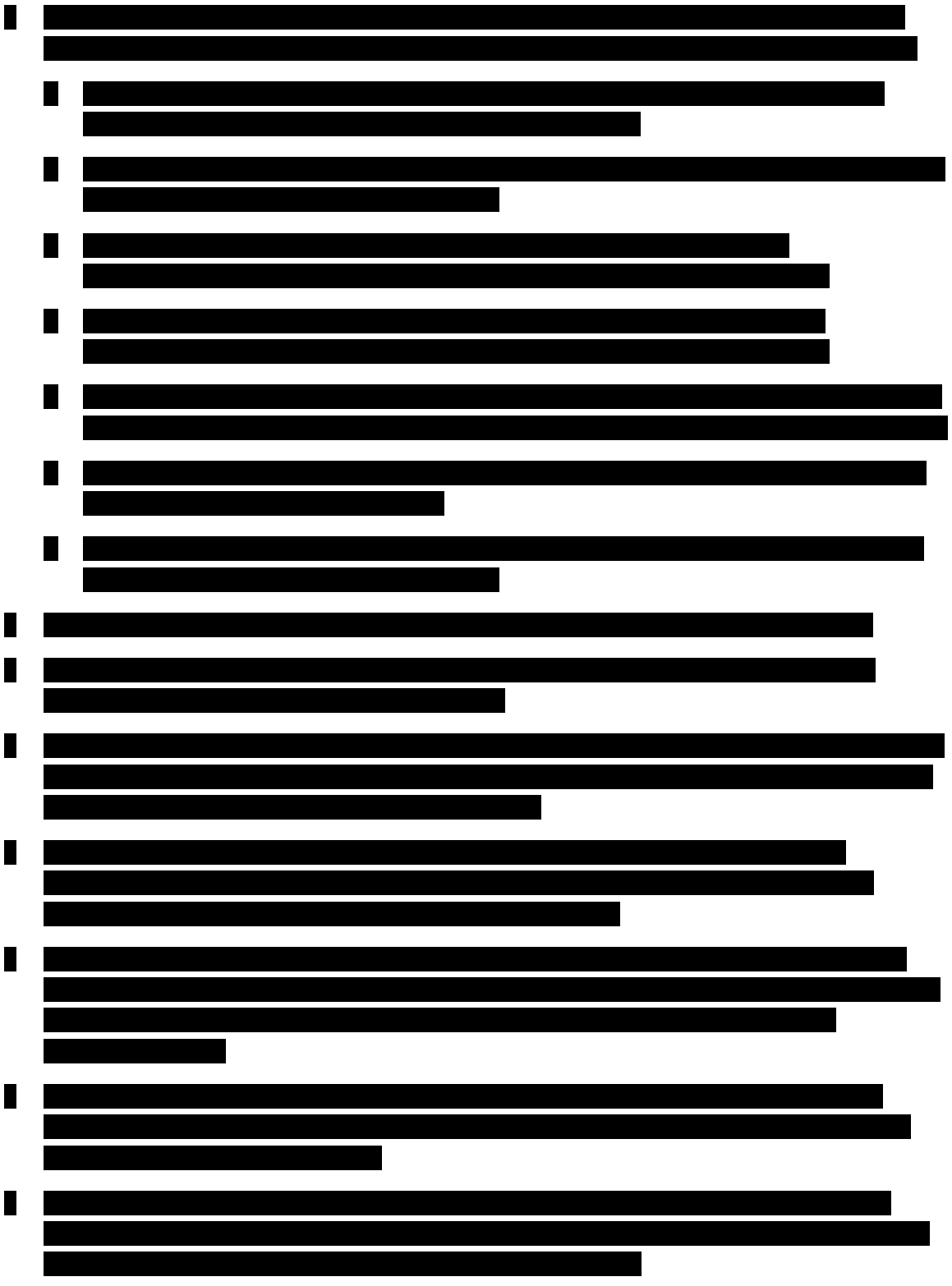
Assess the efficacy of AVXS-101 based on the proportion of patients achieving the ability to stand without support for at least 3 seconds as defined by BSIDv03 Gross Motor Subtest Item #40 at any visit up to 24 months of age.

6.2.2.2. Secondary Objectives (3 copy)

Assess the efficacy of AVXS-101 by demonstrating the ability to walk alone defined as the [redacted] as defined by BSIDv03 Gross Motor Subtest Item #43 at any visit up to 24 months of age.

6.2.2.3. Exploratory Objectives (3 copy)

[redacted]



7. INVESTIGATIONAL PLAN

7.1. Overall Study Design

This is a Phase 3, open-label, single-arm study of a single, one-time dose of AVXS-101 (gene replacement therapy) in patients with SMA who meet enrollment criteria and are genetically defined by bi-allelic deletion of *SMN1* with 2 or 3 copies of *SMN2*. Patients with *SMN1* point mutations or the *SMN2* gene modifier mutation (c.859G>C) may enroll but will not be included in the efficacy analysis sets.

At least 14 patients with bi-allelic deletion of *SMN1* and 2 copies of *SMN2*, and at least 12 patients with bi-allelic deletion of *SMN1* and 3 copies of *SMN2* that are ≤6 Weeks of age **at the time** of gene replacement therapy (Day 1) will be enrolled.

The study includes a screening period, an inpatient gene replacement therapy period, and an outpatient follow-up period. During the screening period (Days –30 to –2), patients whose parent(s)/legal guardian(s) provide informed consent will undergo screening procedures to determine eligibility for study enrollment. Patients who meet the entry criteria will enter the in-patient gene replacement therapy period (Day –1 to Day 2). On Day –1, patients will be admitted to the hospital for pre-treatment baseline procedures. On Day 1, patients will receive a single, one-time IV infusion of AVXS-101 and will undergo in-patient safety monitoring over the next 24 hours. Patients may be discharged 24 hours after the infusion, based on Investigator judgment. During the outpatient follow-up period (Days 3 to EOS at 18 or 24 months of age, dependent upon respective *SMN2* copy number), patients will return at regularly scheduled intervals for efficacy and safety assessments until the EOS when the patient reaches 18 months of age (*SMN2* = 2) or 24 months of age (*SMN2* = 3). After the EOS visit, patients will be monitored for safety for 30 days. Additionally, eligible patients will be invited to participate in a long-term follow up study conducted under a separate protocol.

After dosing, follow-up visits will be conducted according to the Schedule of Assessments (SoA) (Appendix 1). Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose (Day 1). Visits following Day 72 are based on patient's date of birth. The EOS visit for patients with 2 and 3 copies of *SMN2* will be the 18 and 24 months old visits, respectively. However, safety monitoring will continue for 30 days after EOS visit. Any missed visit should be rescheduled as soon as possible within the window described in the SoA. All visits will be scheduled based on a 30-day month calender.

In an attempt to dampen the host immune response to the AAV derived therapy, all patients will receive prophylactic prednisolone (or equivalent glucocorticoid) at approximately 2 mg/kg/day on Day -1, Day 1, and Day 2, and then 1 mg/kg/day starting on Day 3 and until at least 30 days post-AVXS-101 infusion. After 30 days post-AVXS-101 infusion, the dose of prednisolone can be tapered for patients whose gamma glutamyl transferase (GGT), ALT values, and AST values are below the threshold of 2 × Upper Limit Normal (ULN) in accordance with the following treatment guideline: taper from 1 mg/kg/Day to 0.5 mg/kg/Day during Weeks 5 and 6 post-AVXS-101 infusion, then taper to 0.25 mg/kg/Day during Weeks 7 and 8, and then discontinue prednisolone at Week 9.

If the GGT, AST, or ALT values are > 2 × ULN, the dose of prednisolone will be maintained until the GGT, AST, and ALT values decrease below threshold. 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.

Efficacy will be assessed by achievement of the key developmental milestone of functional independent sitting for at least 30 seconds (*SMN2* = 2) at any visit up to 18 months of age; and ability to stand without support for at least 3 seconds (*SMN2* = 3) at any visit up to 24 months of age. Additional developmental milestones will be assessed using the WHO-MGRS and BSIDv03. Safety will be assessed through monitoring AEs, concomitant medication usage, physical examinations, vital sign assessments, cardiac assessments, and laboratory evaluations. The primary efficacy analysis for each *SMN2* copy number cohort will be completed separately at such time that enrollment in the respective cohort is complete and the last patient has discontinued or completed the EOS visit at the respective age (*SMN2* = 2, EOS = 18 months of age; *SMN2* = 3, EOS = 24 months of age).

After the EOS visit, patients will be invited to participate in a long-term follow-up study conducted under a separate protocol. Patients who discontinue prematurely will also be invited to participate in the long-term follow-up study.

In the event that unforeseen catastrophic or other serious situations (such as the COVID-19 pandemic) impact the ability to conduct the study on-site, alternative methods of continuing study assessments may be implemented. Alternative visits include phone calls, virtual contacts through teleconsult or videoconference, or visits by site staff/home nursing providers to the patient's home depending on local regulations, institutional policies, and capabilities of the investigative site. Alternative visits may take place instead of on-site visits until such time that the patientt can safely return to the site. The process for obtaining remote informed consent must be followed if alternative study visits will be conducted (Section 18.3).

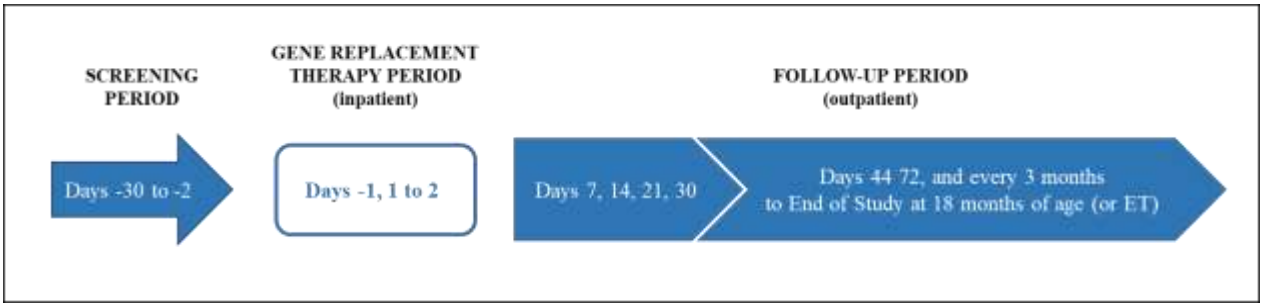
Throughout the study, the scheduling of study visits should adhere to the overall study schedule and visit windows defined in the protocol. For each study visit, all study procedures and assessments should be performed or completed in accordance with the schedule of events. Local institutions or other alternatives may be utilized.

Importantly, in instances in which the final scheduled study visit is not possible to be conducted at the study site, the final scheduled study visit should be performed all or by an alternative visit within the protocol-defined visit window. Critical data that pertain to the primary and secondary endpoints and other information such as adverse events, concomitant medications, use of feeding or ventilatory support, or other safety data that may be obtained remotely should be collected as part of the final study visit and within the protocol-defined visit window.

Procedures such as laboratory and other safety assessments that cannot be performed or obtained as part of a remote visit may be performed separately and preferably within the protocol-defined visit window.

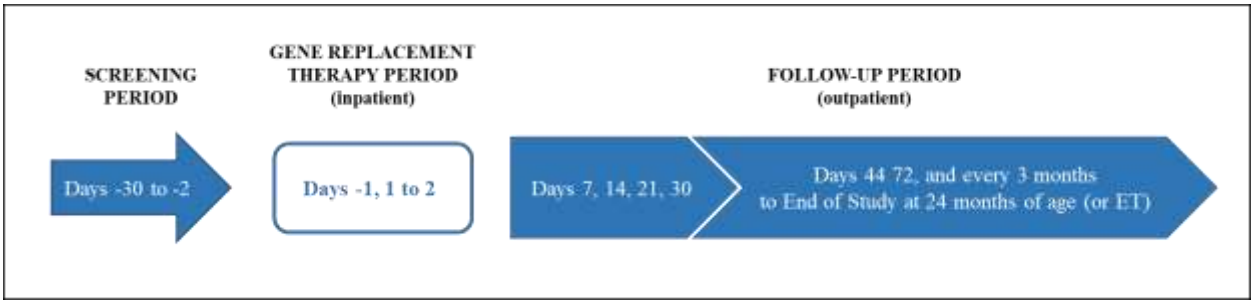
Figure 4 - Study Design

Patients with 2 copies of *SMN2*



Note: After the EOS visit at 18 months of age, patients will be monitored for safety for 30 days after the EOS visit. Additionally, patients will be invited to participate in a long-term follow-up study conducted under a separate protocol.
EOS = End of Study; ET = early termination

Patients with 3 copies of *SMN2*



Note: After the EOS visit at 24 months of age, patients will be monitored for safety for 30 days after the EOS visit. Additionally, patients will be will be invited to participate in a long-term follow-up study conducted under a separate protocol.
EOS = End of Study; ET = early termination

Additionally, a Data Safety Monitoring Board (DSMB)/Data Monitoring Committee (DMC) will review safety data on a quarterly basis. A detailed description of the DSMB/DMC, its role in this trial, and the timing and process of the scheduled reviews will be described in a DSMB/DMC Charter.

7.2. Number of Patients

At least 26 patients meeting the ITT criteria will be enrolled. Sponsor may cap enrollment in one geographic region to ensure appropriate regional patient distribution as necessary.

7.3. Criteria for Study Termination

An independent DSMB/DMC will conduct quarterly and ad hoc reviews of the emerging safety data throughout the study as described in Section 15.

The study may be terminated for the following reasons:

- Development of unacceptable toxicity, defined as the occurrence of any 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.
- 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 SMA who are ≤6 Weeks (≤42 days) of age **at the time** of gene replacement therapy (Day 1) with documented absence of the *SMN1* gene and 2 or 3 copies of the *SMN2* gene will be enrolled in this study. Patients may be of any racial, ethnic, or gender background.

The primary and secondary efficacy analyses will be performed on the population of patients with bi-allelic *SMN1* deletions and 2 or 3 copies of *SMN2* without the *SMN2* gene modifier mutation (c.859G>C). While the study may enroll a small number of patients that don't meet these criteria, the above will comprise the population for evaluation of the primary and secondary endpoints and will be referred to as the Intent-To-Treat (ITT) population hereafter. Patients with *SMN1* point mutations and patients with the *SMN2* gene modifier mutation (c.859G>C) will be evaluated separately as part of additional subgroup analyses.

8.1. Patient Inclusion Criteria

Patients must meet all of the following inclusion criteria:

All patients:

1. Age ≤6 Weeks (≤42 days) at time of dose
2. Ability to tolerate thin liquids as demonstrated through a formal bedside swallowing test
3. Compound muscle action potential (CMAP) ≥2mV at Baseline; centralized review of CMAP data will be conducted
4. Gestational age of 35 to 42 weeks
5. Up-to-date on childhood vaccinations that include palivizumab prophylaxis (also known as Synagis) to prevent RSV infections are also recommended in accordance with the guidance of local health authorities.
6. Able and willing to follow the Consensus Statement for Standard of Care in Spinal Muscular Atrophy (36)
7. Parent(s)/legal guardian(s) willing and able to complete the informed consent process and comply with study procedures and visit schedule
8. Genetic diagnosis as described below, obtained from an acceptable newborn or pre-natal screening test method:

   Patients with 2 copies of *SMN2* (n ≥14)

   - Patients with pre-symptomatic SMA Type 1 as determined by the following features:
     - 2 copies of *SMN2*

   Patients with 3 copies of *SMN2* (n ≥12)

   - Patients with pre-symptomatic SMA Type 2 as determined by the following features:
     - 3 copies of *SMN2*

8.2. Patient Exclusion Criteria

Patients must not meet any of the following exclusion criteria:

1. Weight at screening visit <2 kg
2. Hypoxemia (oxygen saturation <96% awake or asleep without any supplemental oxygen or respiratory support) at the screening visit or for altitudes >1000 m, oxygen saturation <92% awake or asleep without any supplemental oxygen or respiratory support at the screening visit
3. Any clinical signs or symptoms at screening or immediately prior to dosing that are, in the opinion of the Investigator, strongly suggestive of SMA (e.g., tongue fasciculation, hypotonia, areflexia)
4. Tracheostomy or current prophylactic use or requirement of noninvasive ventilatory support at any time and for any duration prior to screening or during the screening period
5. Patients with signs of aspiration/inability to tolerate nonthickened liquids based on a formal swallowing test performed as part of screening or patients receiving any non-oral feeding method
6. Clinically significant abnormal laboratory values (GGT, ALT, and AST, or total bilirubin > 2 × the 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.
7. Patients with any other clinically significant abnormalities in hematology or clinical chemistry parameters as determined by investigator or medical monitor
8. Treatment with an investigational or commercial product, including nusinersen, given for the treatment of SMA. This includes any history of gene therapy, prior antisense oligonucleotide treatment, or cell transplantation.
9. Patients whose weight-for-age is below the third percentile based on World Health Organization (WHO) Child Growth Standards (37).
10. Biological mother with active viral infection as determined by screening laboratory samples (includes human immunodeficiency virus [HIV] or positive serology for hepatitis B or C)
    a. Biological mothers with clinical suspicion of Zika virus that meet Centers for Disease Control and Prevention (CDC) Zika virus epidemiological criteria including history of residence in or travel to a geographic region with active Zika transmission at the time of travel will be tested for Zika virus RNA. Positive results warrant confirmed negative Zika virus RNA testing in the patient prior to enrollment.
11. Serious nonrespiratory tract illness requiring systemic treatment and/or hospitalization within 2 Weeks prior to screening
12. Upper or lower respiratory infection requiring medical attention, medical intervention, or increase in supportive care of any manner within 4 Weeks prior to dosing

13. Severe nonpulmonary/respiratory tract infection (e.g., pyelonephritis, or meningitis) within 4 Weeks before administration of gene replacement therapy or concomitant illness that, in the opinion of the Investigator or Sponsor medical monitor, creates unnecessary risks for gene replacement therapy such as:
    a. Major renal or hepatic impairment
    b. Known seizure disorder
    c. Diabetes mellitus
    d. Idiopathic hypocalciuria
    e. Symptomatic cardiomyopathy
14. Known allergy or hypersensitivity to prednisolone or other glucocorticosteroids or their excipients.
15. Previous, planned or expected major surgical procedure including scoliosis repair surgery/procedure during the study assessment period
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, immunosuppressive therapy within 4 Weeks prior to gene replacement therapy (e.g., corticosteroids, cyclosporine, tacrolimus, methotrexate, cyclophosphamide, IV immunoglobulin, rituximab)
17. AntiAAV9 antibody titer >1:50 as determined by Enzyme-linked Immunosorbent Assay (ELISA) binding immunoassay
    a. Should a potential patient demonstrate AntiAAV9 antibody titer >1:50, he or she may receive retesting inside the 30-Day screening period and will be eligible to participate if the AntiAAV9 antibody titer upon retesting is ≤1:50, provided the <6 Week age requirement at the time of dosing is still met
18. Biological mother involved with the care of the child refuses anti-AAV9 antibody testing prior to dosing
19. Parent(s)/legal guardian(s) unable or unwilling to comply with study procedures or inability to travel for repeat visits
20. Parent(s)/legal guardian(s) unwilling to keep study results/observations confidential or to refrain from posting confidential study results/observations on social media sites
21. Parent(s)/legal guardian(s) refuses to sign consent form

8.3. Patient Withdrawal Criteria

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

- Death
  - Post-mortem tissue/sample collection will be requested for any patient who expires following treatment with AVXS-101 (see Post-mortem Plan in Appendix 2)
- Parent(s)/legal guardian(s) withdraws consent
- Investigator discretion

End of study procedures should be completed within 14 days for any patient who prematurely discontinues the study for any reason, as indicated in Appendix 1. Patients who terminate the study early for reasons other than death will be offered enrollment in a long-term follow-up study.

In instances in which a patient withdraws from the study prior to the last scheduled visit, the investigator should differentiate between factors that are related to a catastrophe (such as the inability to travel, local ordinances or other restrictions, etc.) versus withdrawal of consent.

9. TREATMENT OF PATIENTS

It is the responsibility of the Investigator to ensure the safe storage and administration of gene replacement therapy.

9.1. Description of Product

The biological product is a non-replicating recombinant AAV9 containing the complimentary deoxyribonucleic acid (cDNA) of the human *SMN* gene under the control of the cytomegalovirus (CMV) enhancer/CB promoter. 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. The biological product, called AVXS-101, expresses the human SMN protein in transduced cells.

Table 2 - Investigational Product

	Investigational Product
Product Name:	AVXS-101
Unit Dose	1.1×10^{14} vg/kg
Route of Administration	Intravenous infusion
Physical Description	AVXS-101 is a clear, colorless to faint white solution.

9.2. Prior and Concomitant Medications

Prior and concomitant medications will be captured in the electronic Case Report Form (eCRF) from 2 Weeks prior to administration of gene replacement therapy through the last study visit.

9.2.1. Prophylactic Administration of Prednisolone

An antigen specific T-cell response to the AAV vector was observed in the completed Phase 1 clinical study (AVXS-101-CL-101) investigating AVXS-101 treatment via IV infusion [data on file]. This is an expected response between 2 to 4 Weeks following gene replacement therapy. 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, all patients will receive prophylactic prednisolone at approximately 2 mg/kg/day (or an equivalent dose of another glucocorticoid if prednisolone is unavailable or in the opinion of the investigator prednisolone is not tolerated (38)) on Day -1, Day 1, and Day 2, and then 1 mg/kg/day starting on Day 3 and until at least 30 days post-AVXS-101 infusion. After 30 days post-AVXS-101 infusion, the dose of prednisolone can be tapered for patients whose GGT, ALT values, and AST values are below the threshold of 2 × ULN. To summarize, the overall course of prednisolone should follow the following treatment guideline:

- Day -1, Day 1, and Day 2: 2 mg/kg/day
- Day 3 until at least 30 days post-AVXS-101 infusion: 1 mg/kg/day
- Weeks 5 and 6 post-AVXS-101 infusion: 0.5 mg/kg/day
- Weeks 7 and 8 post-AVXS-101 infusion: 0.25 mg/kg/day
- Week 9 post-AVXS-101 infusion: prednisolone discontinued

Liver function testing should guide each step of the taper, and liver function tests (LFTs) 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 maintained until the GGT, AST, and ALT values decrease below threshold, at which time 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 diabetes, myopathy or neuropathy
- Therapy received with the intent to treat SMA (e.g., nusinersen, valproic acid)
  - Oral β-agonists must be discontinued at least 30 days prior to gene therapy dosing
  - Inhaled βagonists may be used to treat respiratory complications of SMA provided such medications are dosed at clinically appropriate levels
- Any investigational medication other than AVXS-101 is prohibited during the study
- Ongoing immunosuppressive therapy, plasmapheresis, immunomodulators such as adalimumab, or immunosuppressive therapy within 4 Weeks of starting the study (e.g., corticosteroids, cyclosporine, tacrolimus, methotrexate, cyclophosphamide, IV immunoglobulin, rituximab)

Corticosteroid usage following completion of the prednisolone taper is permissible as part of routine clinical management. The use of corticosteroids 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 medical monitor.

9.2.3. Vaccinations

Where feasible, the vaccination schedule should be adjusted appropriately to accommodate the 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 judgement 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 (39, 40).

Vaccinations that include palivizumab (also known as Synagis®) prophylaxis to prevent respiratory syncytial virus (RSV) infections are also recommended (40, 41).

9.3. Treatment Compliance

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

9.4. Randomization and Blinding

This is an open-label study.

10. STUDY PRODUCT MATERIALS AND MANAGEMENT

AVXS-101 is manufactured in accordance with current Good Manufacturing Practices (cGMP).

10.1. Study Product

AVXS-101

10.2. Study Product Dose and Dose Justification

Patients will receive a onetime dose of AVXS-101 at 1.1×10^{14} vg/kg; this dose is equivalent to the dose received by the second dosing cohort in the Phase 1 study via IV infusion administered in the Phase 1 clinical study (AVXS-101-CL-101).

Two doses were studied in the completed Phase 1 clinical study (AVXS-101-CL-101); the higher dose (dose received by the Cohort 2 patients) was chosen for the present study as preliminary data demonstrated both a dose response and significant clinical benefit thus identifying it as the proposed therapeutic dose. In the Phase 1 study, AVXS-101 demonstrated a dose response, with efficacy greater as observed by motor milestone achievement and CHOP INTEND scores at the higher dose (received by Cohort 2) than the lower dose (received by Cohort 1). Direct testing of the actual lot of Investigational Medicinal Product (IMP) used in the AVXS-101-CL-101 study by an improved and more fully qualified analytical method has assigned a value of 1.1×10^{14} vg/kg to the actual dose received by Cohort 2 in this Phase 1 study. The same method has been used to establish an equivalent dose for the Phase 3 IMP. This vg/kg value has been further verified in an improved and more fully qualified SMNΔ7 Mouse Biopotency assay to support a similar extension of mouse life time in direct comparative assessment between the Phase 1 and Phase 3 IMP.

10.3. Study Product Packaging and Labeling

AVXS-101 kits will be labeled with a specific kit number and 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 in the procedure room and processed for destruction and/or return to AveXis, Inc. (AveXis) in accordance with the Pharmacy manual and applicable biohazardous waste guidelines for disposal.

10.5. Study Product Preparation

Preparation of AVXS-101 should be done under sterile conditions by a pharmacist and will arrive at the clinical site ready for infusion.

The total vector genome dose will be calculated based on the patient's body weight.

The dose-delivery syringe will be delivered to the designated dosing location in accordance with the Pharmacy Manual.

10.6. Study Product Administration

AVXS-101 infusion will be administered under sterile conditions in a pediatric intensive care unit (PICU) or other appropriate setting (e.g., interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management. AVXS-101 will be delivered one-time through a venous catheter inserted into a peripheral limb vein (arm or leg) at a dose of 1.1×10^{14} vg/kg. AVXS-101 should be slowly infused over approximately 60 minutes utilizing an infusion set and syringe pump in accordance with the Pharmacy Manual. Vital signs will be continuously monitored throughout the gene replacement therapy infusion as described in Section 12.1.3.

Following administration of gene replacement therapy, patients should remain in the dosing suite or be moved to an appropriate designated setting to ensure close monitoring of vital signs and AEs for a minimum of 24 hours after the start of gene replacement therapy.

10.7. Dose Adjustment Criteria

The study investigates a one-time IV infusion of AVXS-101; no dose adjustments are possible.

10.8. Study Product Accountability

The pharmacist or designee will maintain accurate records of the quantities of AVXS-101 received, dispensed, destroyed, and/or returned to AveXis. 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 return of the used vessel to AveXis or destruction as per the Pharmacy Manual or the site's local standard operating procedure.

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 personal protective equipment, 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 the Sponsor 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 study protocol, or dispose of AVXS-101 in any other manner.

11. ASSESSMENT OF EFFICACY

The primary efficacy endpoint will be achievement of the key developmental milestone of functional independent sitting for at least 30 seconds at any visit up to the 18 months of age study visit for patients with bi-allelic deletions of *SMN1* and 2 copies of *SMN2*. The secondary endpoint of survival, the proportion of patients that have survived and have not required permanent ventilation in the absence of acute illness or perioperatively, will be assessed at 14 months of age. The secondary endpoint of ability to thrive (proportion of patients that have achieved the ability to maintain weight at or above the third percentile without need for non-oral/mechanical feeding support) will be assessed at any visit up to the 18 months of age for these patients. Additional developmental milestones will be assessed using the WHO-MGRS and BSIDv03.

The primary efficacy endpoint will be achievement of key developmental milestone of standing without support for at least 3 seconds at any visit up to the 24 months of age study visit for patients with bi-allelic deletions of *SMN1* and 3 copies of *SMN2*. The secondary efficacy endpoint of achievement of the ability to walk alone defined as [redacted] will be assessed at any visit up to the 24 months of age for these patients. Additional developmental milestones will be assessed using the WHO-MGRS and BSIDv03.

As the two *SMN2* copy number cohorts (2 and 3 copy) described in this protocol have different natural histories and different disease severities, and, appropriate to their differing severities and natural histories will have different primary endpoints and different follow-up time periods in this protocol, each copy number cohort is in essence a separate clinical study. The two studies/cohorts have been combined under a single protocol for administrative efficiency. As such, efficacy will be assessed in each cohort independently of the other.

Efficacy assessments will be performed at the times specified in the Table of Assessments (Appendix 1) and should be the first assessments performed at any scheduled visit.

In the event of unforeseen catastrophe or other, serious situation (such as the COVID-19 pandemic) that occurs during the study, which limits or prevents on-site study visits, alternative methods of data collection pertaining to efficacy assessments may be considered. Critical data that pertain to the primary and secondary endpoints should be collected according to the overall study schedule and within the protocol-defined visit window. The maintenance of the overall visit schedule and visit windows is especially important for the final scheduled study visit, as this may impact the overall interpretation of the evaluation of efficacy. As described in Section 7.1, allowances are made for the collection of data remotely, when necessary.

Video recordings demonstrating the attainment of any new developmental milestones should be obtained within the protocol-defined visit window, including those that are required to be assessed as part of the final study visit or are related to primary or secondary endpoints (e.g, sitting without support). This may include submission of recordings made by the family or others as described in Section 11.3.

Alternative methods of data collection for efficacy assessments depending on local regulations, institutional polices, and capabilities may include:

- developmental/motor efficacy assessments by a qualified clinical evaluator during a visit to the participant´s home

- Home videos or other recordings which demonstrate the achievement of developmental milestones that are specified in the protocol, including those to be assessed at the final scheduled study visit
- use of ventilatory, cough or feeding support by the participant, evaluated during phone calls, virtual contacts (e.g., teleconsult) or visits by site staff/home nursing service to the participant´s home, or during visits by the participant to a local hospital.

11.1. Developmental Milestones

Developmental milestones will be assessed using relevant definitions obtained from the BSIDv03 and WHO-MGRS (Appendix 5) (35), and will be analyzed to assess efficacy (Appendix 3 and Appendix 4). Achievement of each developmental milestone will be determined by qualified site clinical evaluator (physical or occupational therapist, or national equivalent) and confirmed by the central reviewer based on an assessment of the submitted videotapes (Section 11.3). Developmental milestones will be determined as shown in the SoA.

The developmental milestone of sitting independently (BSIDv03 item #26: sits without support for at least 30 seconds) should be assessed until attainment of milestone, regardless of starting point on the scale. **This milestone must be assessed, documented, and video recorded at the 18 months of age visit for patients with 2 copies of *SMN2*, regardless of previous attainment. End of study maintenance of this developmental milestone will be submitted for review by an independent central reviewer.**

The developmental milestones of standing without support for at least 3 seconds and walking alone (BSIDv03 items #40 and #43, respectively) should be assessed until attainment of milestone, regardless of starting point on the scale. **These developmental milestones must also be assessed, documented, and video recorded at the 24 months of age visit for patients with 3 copies of *SMN2*, regardless of previous attainment. End of study maintenance of this developmental milestone will be submitted for review by an independent central reviewer.**

As the Bayley Scales do not necessarily require the child to repeat previously attained developmental milestones, it is essential that each attained milestone be captured on video.

A developmental milestone will be considered achieved when demonstrated by a patient and observed with video capture confirmation during a physical assessment or observed with video as provided by the patient's family and confirmed by a central reviewer. Developmental milestones can be achieved at any point during the study. However, cohort-dependent primary and secondary endpoints should be reconfirmed at the patient's EOS visit at 18 or 24 months of age.

11.2. Motor Function Tests

11.2.1. Bayley Scales of Infant and Toddler Development/Developmental Milestones and World Health Organization Multicentre Growth Reference Milestones

The BSIDv03 (Appendix 3) is a standardized, norm-referenced infant assessment of developmental functioning across 5 domains of cognitive, language, motor, social-emotional, and adaptive behavior. The Bayley Scales will be administered by a qualified clinical evaluator in accordance with the Physical Assessments Manual. The items from the BSIDv03 Gross Motor Subtest defined as developmental milestones for this protocol are specified in Appendix 4.

During the Screening visit, the clinical evaluator will complete an assessment of baseline milestone achievement in accordance with Appendix 3; this assessment must address all milestones/items noted in Appendix 3, Gross Motor Subtest that are at or below the child's baseline function, and be recorded on video. The findings must be documented in the source. Items that are below the baseline level of assessment that are not successfully achieved during the baseline evaluation should be repeated at subsequent visits until successfully performed.

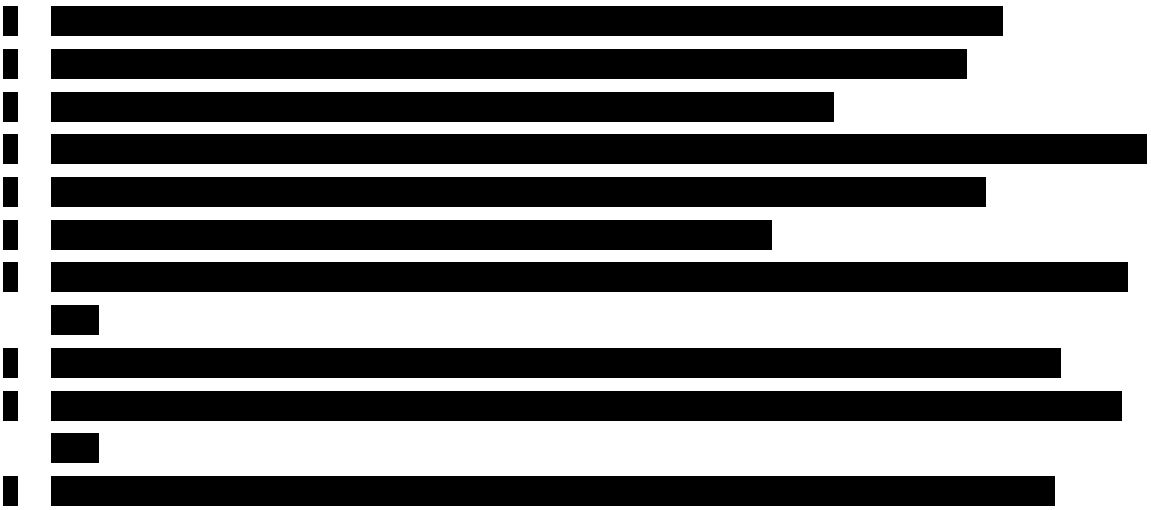
The milestones of sitting independently (item #26) should be assessed, documented and recorded at every subsequent visit, until attainment of milestone, regardless of starting point on the scale.

As the Bayley Scales do not necessarily require the child to repeat previously attained milestones, it is essential that each attained milestone be captured on video.

The gross and fine motor subtests of the motor domain will be administered according to the SoA (Appendix 1).

Each Bayley Scales/WHO MGRS developmental milestone assessment will be video recorded in accordance with the AVXS-101-CL-304 Physical Assessments Manual and will be submitted for review by an independent central reviewer (Section 11.3).

The following developmental milestones will be [redacted] (Appendix 4 and Appendix 5):



11.2.2. CHOP INTEND

The CHOP INTEND (Appendix 6) is a motor function scale developed and validated for use specifically to monitor motor function status and decline amongst children with SMA Type 1, and will be administered by a qualified clinical evaluator (42, 43). The CHOP INTEND scale examines several aspects of motor function, including head control, righting reactions, and trunk movements in supported sitting, supine, and prone positions (Appendix 6). Anti-gravity movements in assisted rolling, ventral suspension, and supported standing will also be measured. Additional information on contractures will also be collected as described in the Physical Assessments Manual.

The CHOP INTEND will be performed according to the SoA for patients with 2 copies of *SMN2* (Appendix 1). For purposes of efficacy assessments, the CHOP INTEND assessment performed at Day -1 will be treated as baseline.

Patients who achieve 3 consecutive CHOP INTEND scores ≥58 will not undergo any additional CHOP INTEND examinations.

Each CHOP INTEND exam will be video recorded in accordance with the AVXS-101-CL-304 Physical Assessments Manual and may be submitted for review by an independent central reviewer (Section 11.3).

11.3. Video Evidence

Clinic assessments (WHO Motor Developmental Milestones, Bayley Scales, CHOP INTEND, developmental milestone assessments) will be video recorded 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, the medical community, and/or in appropriate venues to discuss the results of this clinical study.

Videos demonstrating Developmental Milestones which meet WHO and BSIDv03 criteria will be submitted to an independent, central reviewer for unbiased assessment of developmental milestone achievement. The independent central reviewer will document whether the video displays evidence of having achieved each developmental milestone. The date of developmental 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 study. These videos will be handled in the same manner in which the study-derived videos are handled.

11.4. Compound Motor Action Potential

Peroneal nerve CMAP amplitude will be measured by a qualified electrophysiologist using the procedures as described in the CMAP Manual. CMAP will be measured at screening, every 6 months starting at 6 months of age, and EOS when the patient reaches 18 or 24 months of age (or early termination) (Appendix 1), depending on respective cohort.

The CMAP data will be collected for centralized review and interpretation.

12. ASSESSMENT OF SAFETY

In the event of unforeseen catastrophe or other, serious situation (such as the COVID-19 pandemic) that occurs during the study, which limits or prevents on-site study visits, regular phone or virtual video calls may occur (as per the Schedule of Assessments) for safety monitoring and discussion of the participant´s health status. Visits by site staff/home nursing service to the participant´s home, or visits by the participant to a local hospital, laboratory, or imaging center, for performing certain safety assessments, depending on local regulations and capabilities, can replace on-site study visits, if necessary. Analysis of laboratory samples (e.g., chemistry, hematology, urinalysis) may be performed at a local laboratory, if it is not possible to follow the central laboratory process. Other safety assessments that cannot be conducted by telephone (such as vital signs, weight, length, cardiac testing etc.) may similarly be collected by the methods described above.

12.1. Safety Parameters

Safety parameters include physical examinations, pulmonary examinations, vital signs, weight and length measurements, 12-lead ECGs, 24-hour Holter monitoring, echocardiograms, swallowing tests, laboratory assessments, AE monitoring, and photographs of the infusion site. In general, safety assessments will be performed at the times specified in the SoA (Appendix 1). Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose (Day 1). Visits following Day 72 are based on patient's date of birth.

12.1.1. Demographic/Medical History

Demographic/medical history information will be collected at screening and captured in the eCRF. Information that will be collected includes:

- Familial history of SMA including affected siblings or parent carriers
- Gestational age at birth
- Length/weight/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

Patients are encouraged to follow all routinely scheduled immunizations, as recommended by local health authorities, throughout the study. Vaccinations that include palivizumab prophylaxis (also known as Synagis) to prevent RSV infections are also recommended in accordance with the guidance of local health authorities.

12.1.2. Physical Examinations

Physical examinations will be conducted by the Investigator or designee as specified in the SoA (Appendix 1). The Day 1 physical examination will be performed prior to the start of gene replacement therapy infusion. Physical examinations include a review of the following systems: head, eyes, ears, nose and throat (HEENT), lungs/thorax, cardiovascular, abdomen, musculoskeletal, neurologic, dermatologic, lymphatic, and genitourinary. Specifically, the neurological exam should include detailed, age-appropriate sensory testing (such as examination of proprioceptive, vibratory, tactile and pain sensation) at each visit. Any clinically significant abnormal finding should be recorded as an AE. Further clinical evaluation should be considered as per judgment of the investigator.

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.

12.1.3. Vital Signs/Weight and Length

Vital sign parameters include blood pressure, respiratory rate, pulse, temperature, and pulse oximetry. Vital signs will be obtained as specified in the SoA (Appendix 1). On Day 1, vital signs with the exception of blood pressure will be recorded pre-dose and then continuously monitored as specified in the SoA (Appendix 1). Temperature will be recorded pre- and post-infusion.

Weight and length will be measured at each study visit (as specified in Appendix 1). Screening weight shall be obtained ≤14 days prior to Day 1. On Day 1, weight and length will be measured pre-dose.

12.1.4. Electrocardiogram

A 12-lead ECG will be performed as specified in the SoA (or early termination) (Appendix 1). Additional ECG monitoring will be at the discretion of the Investigator as per local institutional guidelines.

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.

12.1.5. Echocardiogram

A standard transthoracic echocardiogram will be performed as specified in the SoA (or early termination) (Appendix 1).

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.

12.1.6. Twenty-Four Hour Holter Monitoring

Twenty-four-hour Holter monitoring will be performed as specified in the SoA (Appendix 1). 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 or designee within 72 hours of data upload. AveXis will be notified of any safety concerns from the centralized review.

12.1.7. Pulmonary Examinations

Pulmonary examinations will be performed by a pulmonologist or appropriate individual as per standard institutional practice as specified in the SoA (Appendix 1). Prior to study entry, a pulmonologist or appropriate individual as per standard institutional practice will review and document lack of requirement for ventilator usage in the 2 Weeks prior to screening.

Patients may be fitted with non-invasive ventilatory support at the discretion of the pulmonologist (or appropriate individual as per standard institutional practice 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 ventilatory support at any time during the study, the usage should be recorded in eCRF. Patients requiring non-invasive ventilatory support will be asked to bring their machine(s) to each study visit.

12.1.8. Swallowing Test

A standard bedside, non-barium (unless required per institutional policy), swallowing test will be performed as specified in the SoA (Appendix 1) to determine if the patient has signs of aspiration for consistencies tested during the assessment. The swallowing test at Screening can be performed at the investigator site. If the test is positive for aspiration, there may be a recommendation for the patient to use an alternate method to oral feeding for the duration of the study at the determination of the Investigator and treating clinician.

12.1.9. Photographs of Infusion Site

Photographs will be taken of the infusion site as specified in the SoA (Appendix 1) to monitor healing of the infusion site. Day 1 infusion site photograph will be performed after gene replacement therapy infusion. 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 study.

12.1.10. Laboratory Assessments

Blood samples will be collected at each scheduled visit as specified in the SoA (Appendix 1) and in accordance with laboratory manuals provided for this study. Any clinically significant laboratory value will be repeated at the discretion of the Investigator.

In most instances, blood samples will be collected and shipped to a central laboratory; however, at the discretion of the Investigator, samples may be processed locally for emergent safety monitoring or other logistical or technical reasons that warrant samples to be processed locally. Samples for laboratory tests required during the in-patient AVXS-101infusion period will be collected and processed by the investigative site's local laboratory to ensure receipt of results prior to discharge. Local laboratory tests should assess the same analytes listed in the protocol and include normal ranges appropriate for the age of the patient.

Table 3 - Total Blood Volume: Patient

Visit	Tests	Total Volume (mL)
Screening[c]	Hematology, chemistry, CK-MB or troponin I[b,d,f], genetic re-confirmation testing	5.6[g]
Day 2	Hematology, chemistry	2.3
Day 7	Hematology, chemistry, CK-MB or troponin I	3.6
Day 14	Chemistry	1.3[e]
Day 21	Hematology, chemistry	2.3
Day 30	Chemistry, CK-MB or troponin I	2.6
Day 44	Chemistry	1.3[e]
Day 60	Hematology, chemistry	2.3
Day 72	Chemistry	1.3[e]
Month 3	Hematology, chemistry	2.3
Quarterly	Hematology, chemistry, CK-MB or troponin I	3.6
End of Study/ET	Hematology, chemistry, CK-MB or troponin I	3.6
Maximum Total Volume for Study[a]: patients with 2 copies of *SMN2*		**42.9**
Maximum Total Volume for Study[a]: patients with 3 copies of *SMN2*		**50.1**

CK-MB = Creatine kinase isoenzyme; EOS = End of Study; ET = early termination

[a.] Patients will have different numbers of monthly visits, depending on the number of copies of SMN2. Maximum total volume based on a completion of all blood draws with maximum volume at each visit, provided mother virus and anti-AAV9 serology is not positive at screening requiring additional testing

[b.] Patients will also have a 1 mL sample collected for baseline anti-AAV9 antibody titer if the biological mother's result is >1:50 or if biological mother is not available for testing

[c.] Screening labs must be completed within 14 days before AVXS-101 dosing

[d.] An additional 1 mL of blood would be required at screening for patients enrolled in regions where newborn screening results do not include *SMN2* copy number

[e.] All sites to collect chemistry at Days 14, 44, and 72.

[f.] CK-MB or troponin-I will be collected at Screening, at the Day 7 and Day 30 visit, and every three months starting at the 6 months of age visit through and including the EOS visit. Patients screened and dosed before IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 will be tested for CK-MB throughout the study. Patients screened before IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 but dosed afterwards, will be tested for CK-MB at screening but for Troponin I at baseline and all subsequent visits. Patients screened after IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 will be tested for Troponin I from screening onwards. The pre-and post-dose cardiac laboratory analytes must match.

[g.] Troponin I requires 0.3 mL less blood than CK-MB, thus total blood volumes will be less for participants receiving troponin I testing.

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 labs: Chemistry → Hematology → CK-MB (or troponin I)
2. Serum antibody to AAV9, if required
3. Genetic re-confirmation testing

Table 4 - Total Blood Volume: Biological Mother

Visit	Tests	Total Volume (mL)
Screening	Viral testing, immunology sample (AAV9 Ab only)	9.8
	Maximum Total Volume for Study	**9.8**

12.1.10.1. Hematology

Hematology analysis will include a complete blood count with differential and platelet count. Samples will be collected and shipped in accordance with the laboratory manual provided by the central laboratory. Blood samples for hematology analysis will be collected as specified in SoA (Appendix 1).

Immediate/same-Day hematology analyses required during in-patient dosing, as determined by the Investigator, and on Day 2 prior to discharge, will be performed as per investigational site standard procedures at the local laboratory. Investigators will receive hematology results from all other study visits from the central laboratory.

12.1.10.2. Blood Chemistry

Samples will be collected and shipped in accordance with the laboratory manual provided by the central laboratory. Blood samples for chemistry analysis will be collected at scheduled visits in accord with (Appendix 1).

Immediate/same-Day chemistry analyses required during in-patient dosing, as determined by the Investigator, and on Day 2 prior to discharge, 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 CK
- Creatinine

- BUN
- Electrolytes (potassium, chloride, CO2, calcium, inorganic phosphorus, sodium)
- Alkaline phosphatase

Creatine kinase isoenzyme (CK-MB), or troponin I, will be collected as specified in SoA (Appendix 1).

- Patients screened and dosed before IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 will be tested for CK-MB throughout the study.
- Patients screened before IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 but dosed afterwards, will be tested for CK-MB at screening but for Troponin I at baseline and all subsequent visits.
- Patients screened after IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 will be tested for Troponin I from screening onwards. The pre- and post-dose cardiac laboratory analytes must match.

12.1.10.3. Urinalysis

Urine samples will be collected in accordance with the laboratory manual provided by the central laboratory at study visits in accord with (Appendix 1). Immediate/same-Day urinalysis required during in-patient dosing, as determined by the Investigator, and on Day 2 prior to discharge, 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 Cell
- White Blood Cell
- Squamous epithelial cells
- Casts
- Crystals

- Bacteria
- Yeast

12.1.10.4. Biological Mother Infectious Disease Testing

The administration of an AAV vector has the risk of causing immune-mediated hepatitis. For patients with HIV, hepatitis B or C, or Zika virus, administration of AAV vector may represent an unreasonable risk; therefore, negative results for the biological mother must be confirmed at screening, prior to treatment. If biological mother is not reasonably available, blood samples must be collected from the patient. These samples will be collected at screening (Appendix 1) and shipped in accordance with the laboratory manual provided by the central laboratory.

Zika testing will only be required for biological mothers with clinical suspicion of Zika virus that meet CDC Zika virus epidemiological criteria including history of residence in or travel to a geographic region with active Zika transmission at the time of travel.

12.1.10.5. Blood for Diagnostic Re-Confirmation Testing

A blood sample will be collected during the screening visit and shipped to the central laboratory in accordance with the laboratory manual for reconfirmation of *SMN1* deletions/mutations, *SMN2* copy number, and absence of exon 7 gene modifier mutation (c.859G>C). Only genetic results from the central laboratory will be used to determine patients' inclusion in the ITT population; this will ensure consistency in diagnostic testing practices.

Results of the diagnostic re-confirmation testing are not required prior to enrollment/dosing; however, this must be completed during screening with results received prior to enrollment/dosing for patients in regions where newborn screening results do not include *SMN2* copy number.

12.1.10.6. AAV9 Antibody Screen in 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 breastmilk. 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 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. Patients whose biological mothers test positive for antibodies to AAV9 will have blood drawn for screening of anti-AAV9 antibodies. Patients who do not have a biological mother available to screen for antibodies to AAV9 will have blood drawn for screening of anti-AAV9 antibodies.

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 casually 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 (related or unrelated) that occur after signing of the informed consent through the last study visit will be collected and recorded in the eCRF.

All AEs will be classified in accordance with the CTCAE version 4.03 outlined in Table 5.

Table 5 - 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 self-care ADL.[b]
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE.

Source: Common Terminology Criteria for Adverse Events (version 4.03) (44).
[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.

Additionally, a DSMB/DMC will review safety data on a quarterly basis. A detailed description of the DSMB/DMC, its role in this trial, and the timing and process of the scheduled reviews will be described in a DSMB/DMC Charter.

13.1.2. Serious Adverse Event

An SAE is an AE occurring during any study phase (e.g., baseline, treatment, washout, or follow-up), and at any dose of the investigational product, or comparator 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
- 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 (related and unrelated) that occur after signing of the informed consent through 30 days after the last study visit must be collected and recorded on forms provided by the Contract Research Organization within 24 hours of the site becoming aware.

13.1.3. Adverse Events of Special Interest

The following are considered AEs of special interest (AESIs):

- Hepatotoxicity
- Thrombocypenia
- Cardiac AEs
- Sensory abnormalities suggestive of ganglionopathy

All AESIs (related or unrelated) that occur after signing of the informed consent through 30 days after the last study visit, irrespective of grade, should be collected and recorded on forms provided by the Contract Research Organization within 24 hours of the site becoming aware.

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 or 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" then the event will be considered related to the investigational product for the purposes of expedited regulatory reporting.

13.3. Recording Adverse Events

Adverse events spontaneously reported by the patient 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. Information about AEs (related and unrelated) after signing of the informed consent form through the last study visit are to be collected. Serious adverse event information will be collected after signing of the informed consent form through 30 days after 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 during Visit 2]), resolution (date [and time if start date during Visit 2]), intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study.

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 after signing of the informed consent through 30 days after 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 or designee 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 e-mail to AveXis or designee. Note: elective procedures or minor surgeries where hospitalization is required should not be reported as SAEs.

Additional follow-up information, if required or available, should all be faxed or e-mailed to AveXis or designee 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 or IEC of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, product-related events (7/15 Day Safety Reports) that occur during the clinical study. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

13.4.1. Reporting to Regulatory Authorities

All SUSARs will be reported to regulatory authorities within the 15-day expedited timeline. Fatal/life-threatening suspected events will be reported according to the 7-day timeline.

14. STATISTICS

This section summarizes key aspects of the analysis plan including definitions of primary, secondary, and exploratory efficacy endpoints and safety endpoints, and the methods to be used to test the primary effectiveness hypothesis. Additional details regarding methods for the final data analysis will be provided in a separate Statistical Analysis Plan (SAP). The SAP will detail all analyses and data displays and will be executed according to Standard Operating Procedures in a controlled environment.

This is a Phase 3 study assessing the efficacy and safety of AVXS-101. The primary efficacy analysis for each *SMN2* copy number cohort will be completed separately at such time that enrollment in the respective cohort is complete and the last patient has completed the EOS visit at the respective age (*SMN2* = 2, EOS = 18 months of age; *SMN2* = 3, EOS = 24 months of age). Details of all analyses will be contained within the SAP.

The primary and secondary efficacy analyses will be performed on the populations of patients with bi-allelic *SMN1* deletions and 2 or 3 copies of *SMN2* without the *SMN2* gene modifier mutation (c.859G>C). The above will comprise the population for evaluation of the primary and secondary endpoints and will be referred to as the ITT populations hereafter. Study power is based upon efficacy analysis of these subpopulations. Patients with *SMN1* point mutations or the *SMN2* gene modifier mutation (c.859G>C) will be evaluated separately as part of additional subgroup analyses.

14.1. Study Endpoints and Populations

14.1.1. Study Endpoints

14.1.1.1. Primary Efficacy Endpoint

Primary Efficacy Endpoint for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

- [redacted]

Primary Efficacy Endpoint for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

- Proportion of patients achieving the ability to stand without support for at least 3 seconds at any visit up to 24 months of age

14.1.1.2. Secondary Efficacy Endpoint

Secondary Efficacy Endpoints for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

- Proportion of patients that have survived and have not required permanent ventilation in the absence of acute illness and perioperatively, assessed at 14 months of age
- Proportion of patients that have achieved the ability to maintain weight at or above the 3rd percentile without need for non-oral/mechanical feeding support at any visit up to 18 months of age

Secondary Efficacy Endpoints for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

- Proportion of patients demonstrating the ability to walk alone defined as [redacted] at any visit up to 24 months of age

14.1.1.3. Exploratory Endpoints

Exploratory Efficacy Endpoints for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

[redacted]

[redacted]

Exploratory Efficacy Endpoints for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

[redacted]

[redacted]

14.1.1.4. Safety Endpoints

Assessment of the safety and tolerability of AVXS-101 treatment includes evaluation of AEs, laboratory data, vital signs, and concomitant medications.

14.1.2. Statistical Analysis Populations

14.1.2.1. Intent-to-Treat Population (ITT)

The ITT population will consist of all enrolled patients with bi-allelic *SMN1* deletions and 2 or 3 copies of *SMN2* without the *SMN2* gene modifier mutation (c.859G>C) who receive AVXS-101. Patients will be analyzed according to the assigned *SMN2* copy number cohort. All efficacy analyses will be conducted using the ITT population as the primary population, unless specified otherwise.

While the study may enroll a small number of patients with *SMN1* point mutations or patients who are positive for the *SMN2* gene modifier mutation (c.859G>C), the population described as the ITT Population above will comprise the population for evaluation of the primary and secondary endpoints.

14.1.2.2. Efficacy Completers Population

The efficacy completers analysis population is a subset of the ITT population and will consist of all patients who are given an AVXS-101 injection and who complete an EOS visit relevant for their assigned cohort (based upon copy number) following the AVXS-101 dose procedure. Patients who terminate early due to other reasons will not be included in the efficacy completers population.

14.1.2.3. All Enrolled Population

The all enrolled population will consist of all patients enrolled (i.e., completed the informed consent process) who receive AVXS-101. Unless specified otherwise, this set will be used for patient listings and for summaries of patient disposition.

14.1.2.4. Safety Population

The safety analysis population will consist of all patients who are given an AVXS-101 injection. The safety population will be used for all analyses of safety endpoints and for the presentation of patients in all patient listings.

14.2. Sample Size Calculation

At least fourteen (14) patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2* and at least 12 patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2* will be enrolled.

One natural history study population is available for comparison with the study population. Distinct control populations drawn from the PNCR study will be used as a comparison for the secondary endpoint of survival for the cohort of patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*, and for the primary and secondary endpoints for the cohort of patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*. Exploratory endpoints will utilize PNCR (2 or 3 copies of *SMN2*) cohorts as appropriate.

The PNCR Natural History dataset (22) is drawn from a large natural history study performed on a large cohort of 337 patients with any form of SMA followed at 3 large, internationally recognized tertiary medical centers with significant expertise in the management of SMA [redacted] Previously identified patients followed in PNCR site clinics and newly diagnosed patients were enrolled. All eligible patients were offered participation in the PNCR study. Study visits were scheduled at baseline and at 2, 4, 6, 9, and 12 months and every 6 months thereafter. The SMA standard of care guidelines published in 2007 were used as a basis for providing uniform care among the study sites.

14.2.1. Patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*

The primary efficacy endpoint for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2* will be the proportion of patients who achieve the ability to sit without support for at least 30 seconds at any visit up to the 18 months of age study visit. Based upon two widely cited natural history studies of the disease (NeuroNext (45), PNCR (22)), it is expected that no patient meeting the study entrance criteria (*SMN2* copy number of 2 without the *SMN2* gene modifier mutation (c.859G>C)) would be expected to attain the ability to sit without support.

The primary efficacy endpoint hypothesis for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

$$\mathbf{H_0}: p_{AVXS-101} \leq 0.1\%$$
$$versus\ the\ alternative$$
$$\mathbf{H_a}: p_{AVXS-101} > 0.1\%,$$

where p is the proportion of functional independent sitting for at least 30 seconds at any visit up to the 18 month study visit.

Based upon data from the completed AVXS-101-CL-101 study, we expect at least 60% of treated patients with 2 copies of *SMN2* to achieve the ability to sit without support for at least 30 seconds. With this efficacy, a sample size of 14 patients would provide power of >90% to detect a significant difference compared with a rate of 0.1% (in lieu of zero) with $\alpha = 0.025$ using a 1-sided exact test for a binomial proportion.

14.2.2. Patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*

The primary efficacy endpoint for patients with 3 copies of *SMN2* will be the proportion of patients who achieve the ability to stand without support for at least 3 seconds at any visit up to the 24 months of age study visit.

The natural history comparison dataset drawn from the PNCR will include all patients with SMA of any type who provided sufficient records for evaluation and met the basic entry criteria for this cohort of the AVXS-101-CL-304 study (3 copies of *SMN2*) and had at least one study visit. The *SMN2* modifier mutation (c.859G>C) described by Prior and colleagues (16) was not assessed in the PNCR study cohort.

Based upon this approach, patient-level data from a cohort of 81 patients drawn from the PNCR Network natural history study of SMA will serve as a "population-matched" control cohort. This comparison cohort encompasses all 81 patients enrolled in the PNCR study who met the criteria of having 3 copies of *SMN2*. Of this cohort, 19/81 (23.5%) of the natural history cohort attained the ability to stand alone (defined as achieving a score of 2 on item #19 of the Hammersmith Functional Motor Scale – Expanded); 17/81 (21%) attained the ability to walk alone (defined as achieving a score of 2 on item #20 of the Hammersmith Functional Motor Scale – Expanded).

The primary efficacy endpoint hypothesis for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

$$\mathbf{H_0}: p_{AVXS-101} = p_{HISTORICAL-PNCR}$$
$$versus\ the\ alternative$$
$$\mathbf{H_a}: p_{AVXS-101} \neq p_{HISTORICAL-PNCR},$$

where p is the proportion of patients who achieve the ability to stand without support for at least 3 seconds at any visit up to the 24 months of age study visit.

Based upon the patient-level data available from the PNCR dataset, 23.5% of patients with *SMN2*=3 who meet the study criteria achieved the ability to stand without support. Extrapolating from the experience from the AVXS-101 Phase 1 study in infants with SMA Type 1, we expect 85% of treated patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2* to achieve the ability to stand 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 Fisher exact test.

14.3. Efficacy Analysis

14.3.1. General Considerations

Primary and secondary efficacy analyses will be based on those patients with bi-allelic deletion mutations of *SMN1*; study cohorts will be based upon *SMN2* copy number, with children with 2 and 3 copies of *SMN2* subject to separate analysis. These analyses are to test the superiority of AVXS-101 to the results from the natural observation study (PNCR) as described above.

Two cohorts of patients (i.e., 2-copies, 3-copies) are evaluated separately in this protocol. Hence, family-wise type I error is controlled within each population cohort, as each cohort is considered its own experiment.

14.3.2. Primary and Secondary Efficacy Analysis for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*

14.3.2.1. Primary Efficacy Endpoint for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*

The primary efficacy endpoint hypothesis for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

$$\mathbf{H_0}: p_{AVXS-101} \leq 0.1\%$$
$$versus\ the\ alternative$$
$$\mathbf{H_a}: p_{AVXS-101} > 0.1\%,$$

where p is the proportion of [redacted] at any visit up to the 18 month study visit.

Testing for functional independent sitting on the cohort of patients with 2 copies of *SMN2* will first be performed using 1-sided exact binomial test with $\alpha = 0.025$. Only if the null hypothesis of equality in proportion of functional independent sitting is rejected at $p<0.025$, will the result be considered statistically significant.

14.3.2.2. Secondary Efficacy Endpoints for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*

The first secondary efficacy hypothesis for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2* to be tested is:

$$\mathbf{H_0}: p_{AVXS-101} = p_{HISTORICAL-PNCR}$$
$$versus\ the\ alternative$$
$$\mathbf{H_a}: p_{AVXS-101} \neq p_{HISTORICAL-PNCR},$$

where p is the proportion surviving and not requiring ventilation at 14 months of age.

Testing for the proportion of patients that have survived and not required permanent ventilation will only be conducted if the result for the functional independent sitting endpoint is statistically significant. Survival will be compared with the control cohort with $\alpha = 0.05$ using a two sample 2-sided Fisher exact test, comparing to patients from natural observational study (PNCR), and the result will be considered statistically significant if $p<0.05$.

The second secondary efficacy endpoint hypothesis for patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2*:

$$\mathbf{H_0}: p_{AVXS-101} \leq 0.1\%$$
$$versus\ the\ alternative$$
$$\mathbf{H_a}: p_{AVXS-101} > 0.1\%,$$

where p is the proportion of patients who have achieved the ability to maintain weight at or above the third percentile without need for non-oral/mechanical feeding support at any visit up to 18 months of age (ability to thrive). Only if the primary endpoint and the first secondary endpoint (survival) for patients with 2 copies of *SMN2* as described above meet statistical significance will this second secondary endpoint of ability to thrive be deemed potentially statistically significant. Testing for ability to thrive for the cohort of patients with 2 copies of *SMN2* will be performed using 1-sided exact binomial test with $\alpha = 0.025$. Only if the null hypothesis of equality in proportion of survival is rejected at $p<0.05$, will the result be considered statistically significant. This hierarchy approach strongly protects against Type I error within the 2 copy cohort.

14.3.3. Primary and Secondary Efficacy Analysis for patients with bi-allelic SMN1 deletions and 3 copies of SMN2

14.3.3.1. Primary Efficacy Endpoint for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*

The primary efficacy endpoint hypothesis for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

$$\mathbf{H_0}: p_{AVXS-101} = p_{HISTORICAL-PNCR}$$
$$versus\ the\ alternative$$
$$\mathbf{H_a}: p_{AVXS-101} \neq p_{HISTORICAL-PNCR},$$

where p is the proportion of patients who achieve the ability to stand without support for at least 3 seconds at any visit up to the 24 months of age study visit.

The proportion of patients that stand without support for at least 3 seconds endpoint for the cohort with 3 copies of *SMN2* will be compared with the control cohort with $\alpha = 0.05$ using a 2-sided two sample Fisher exact test. The result will be considered statistically significant if $p<0.05$.

14.3.3.2. Secondary Efficacy Endpoint for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*

The secondary efficacy endpoint hypothesis for patients with bi-allelic *SMN1* deletions and 3 copies of *SMN2*:

$$\mathbf{H_0}: p_{AVXS-101} = p_{HISTORICAL-PNCR}$$
$$versus\ the\ alternative$$
$$\mathbf{H_a}: p_{AVXS-101} \neq p_{HISTORICAL-PNCR},$$

where p is the proportion of patients who achieve the ability to walk alone at any visit up to the 24 months of age study visit.

The secondary efficacy endpoint for the cohort with 3 copies of *SMN2* will be compared with the control cohort with $\alpha = 0.05$ using a two sample 2-sided Fisher exact test. Only if the primary endpoint for patients with 3 copies of *SMN2* (standing without support) is statistically significant will the secondary endpoint for patients with 3 copies of *SMN2* (walk alone) be assessed as potentially significant. This hierarchy approach strongly protects against Type I error within the 3-copy cohort.

14.4. Safety Analysis

Safety will be assessed through the incidence and toxicity grade of AEs, vital sign assessments, cardiac assessments, laboratory evaluations (chemistry, hematology, urinalysis, immunology), physical examinations, and use of concomitant medications. Adverse events will be coded in accordance with the most current version of the MedDRA coding dictionary.

Safety analyses will be conducted on safety population and summarized by cohort and overall.

14.4.1. Hy's Law Criteria

In order to assess hepatotoxicity, all elevations in liver transaminases are evaluated using Hy's Law criteria as these help to determine the risk of drug-induced liver injury. All elevations in liver transaminases will be assessed against these criteria. Hy's Law cases have the following three components:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo;
2. Among trial patients showing such aminotransferase (AT) elevations, often with ATs much greater than 3×ULN, one or more patients also shows elevation of serum total bilirubin (TBL) to ≥2×ULN, without initial findings of cholestasis (elevated serum ALP);
3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C, pre-existing or acute liver disease, or another drug capable of causing the observed injury (46).

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 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 study and will act in an advisory capacity to AveXis. A detailed description of the DSMB/DMC, its role in this study, 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 study. 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 study, a modification to the study, 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.

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 case report forms, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the case report forms 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, electronic medical records)
- 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 and those SAEs that met criteria for reporting have been forwarded to the IRB/IEC.

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

In the event of unforeseen catastrophe or other, serious situation (such as the COVID-19 pandemic) that occurs during the study, which limits or prevents on-site visits, remote site monitoring visits may be conducted. This should not include remote source data verification (SDV), unless in exceptional circumstances, depending upon local regulations and prior informed consent having been obtained from participating patients.

16.2. Audits and Inspections

Authorized representatives of AveXis, a regulatory authority, an IEC or an IRB 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/Genetically Modified Organisms (GMO) Committee/Competent Authority for Biosafety

As this study involves gene therapy, the Principal Investigator must obtain approval/favorable opinion for the investigation from a designated institutional, local, or independent biosafety committee/GMO Committee/Competent Authority for Biosafety in accordance with institutional, local, regional, and or national requirements and/or guidelines.

16.4. Institutional Review Board/Institutional Ethics Committee

The Principal Investigator must obtain IRB/IEC approval for the investigation (Section 18.1). Initial IRB/IEC 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.

17. QUALITY CONTROL AND QUALITY ASSURANCE

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

Noncompliance with the protocol, ICH, GCP, or local regulatory requirements by an Investigator, site staff, or representatives of the Sponsor will lead to prompt action by the Sponsor 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 or IEC, as appropriate. The Investigator must submit written approval to AveXis before he or she can enroll any patient into the study.

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

The Principal Investigator is also responsible for providing the IRB or IEC 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 or IEC according to local regulations and guidelines.

Initial IRB/IEC 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 (Appendix 7) and are consistent with ICH/GCP, applicable regulatory requirements.

18.3. Written Informed Consent

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

The 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 parent(s)/legal guardian(s).

There will be 3 informed consent forms:

- Parent(s)/legal guardian(s) informed consent form
- Biological mother baseline AAV9 antibody and virus/serology screening informed consent form
- Post-mortem tissue/organ sample collection informed consent form (Appendix 1); if the parent(s)/legal guardian(s) decline an autopsy, it will not prevent the patient from participating in the study)

In the event of unforeseen catastrophe or other, serious situation (such as the COVID-19 pandemic) that occurs during the study, the ability to obtain a standard written informed consent may be challenged due to limits that prevent an on-site visit. In this situation, the Investigator may conduct the informed consent discussion remotely (e.g., telephone, videoconference). Guidance issued by local regulatory bodies on this aspect prevail and must be implemented and appropriately documented (e.g., the presence of an impartial witness, sign/dating separate informed consent forms (ICFs) by trial participant and person obtaining informed consent; a video or other recording of the consent process and affirmation of consent may be considered to supplement the documentation of obtaining consent, if allowable by local regulations etc.).

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 virus/serology results, a check list of inclusion and exclusion criteria, product administration, and a record of sample collection, hemodynamic measurements, clinical assessments, AEs, and final evaluation.

Full date of birth will be recorded to determine satisfaction of inclusion criterion (age ≤6 Weeks (≤42 days) at time of dose).

Electronic CRFs will be used in this study. The eCRF will be electronically signed and dated by the Principal Investigator or 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 included in ICH shall be followed.

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

20. PUBLICATION POLICY

The Investigator is obliged to provide the Sponsor with complete test results and all data derived by the Investigator from the study. During the study, only the Sponsor 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 the Sponsor.

The Sponsor 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 the Sponsor or the Sponsor's 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 or her site.

The Investigator will provide the Sponsor 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. The Sponsor shall inform the Investigator in writing of any changes or deletions in such presentation or publication required to protect the Sponsor's 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 the Sponsor has notified the institution or the Investigator in writing that such proposed publication or presentation discloses the Sponsor's confidential and proprietary technical information. Further, upon the request of the Sponsor, the Investigator will delay the publication or presentation for an additional 90 days to permit the Sponsor to take necessary actions to protect its intellectual property interests.

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22. APPENDICES

APPENDIX 1. SCHEDULE OF ASSESSMENTS

Study Period	Screening		Gene Replacement Therapy (In-patient)			Follow-up (Outpatient)										Clarification
Study Day	**–30 to –2**	**–14 to –2**	**-1**	**1**	**2**	**7**	**14**	**21**	**30**	**44**	**60**	**72**			**End of Study**	Day 1 assessments will be performed prior to the start of gene replacement therapy infusion. Day 7, 14, 21, 30, 44, 60, and 72 visits are timed based on the Day of AVXS-101 dose (Day 1). Visits on Study Days 44 and 72 are for blood chemistry only.
Months of Age													**3[a]**	**6, 9, 12, 15, 21**	**18, 24, (or ET)**	Please see footnote "a" at the bottom of the Appendix 1 table for guidance on scheduling visits for Study Days 44, 60, 72, and 3 Months of Age.
Window						**± 2 days**					**± 7 days**			**± 14 days**	**0–14 days**	The End of Study visit must occur within 0 to 14 days after the date on which the patient reaches 18 or 24 months of age (or ET), depending on the respective cohort. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose. Visits following Day 72 are based on the patient's date of birth.
Informed Consent	X															See Section 18.3.
Prophylactic Prednisolone			X	X	X	X	X	X	X	(X)	(X)					See Section 9.2.1. Prednisolone to be given 24 hours prior to scheduled AVXS-101 dosing and continued as per protocol. (X): Taper prednisolone per protocol between 30 and 60 days after AVXS-101 treatment.
AVXS-101 Infusion				X												See Section 10.6.
Bayley Scales (Fine and Gross Motor Assessment with video)	X								X		X		X	X	X	The Bayley Gross and Fine Motor Subtests are performed at Screening, Day 30 post-dose, Day 60 post-dose, and then every 3 months from 3 months of age visit. See Sections 11.1, 11.2.1, and 11.3.
BSIDv03/WHO Developmental	X								X		X		X	X	X	Developmental milestones will be assessed as defined by BSIDv03 and WHO Multicentre Growth

Study Period	**Screening**		**Gene Replacement Therapy (In-patient)**			**Follow-up (Outpatient)**									**Clarification**
Study Day	**–30 to –2**	**–14 to –2**	**-1**	**1**	**2**	**7**	**14**	**21**	**30**	**44**	**60**	**72**			**End of Study**	Day 1 assessments will be performed prior to the start of gene replacement therapy infusion. Day 7, 14, 21, 30, 44, 60, and 72 visits are timed based on the Day of AVXS-101 dose (Day 1). Visits on Study Days 44 and 72 are for blood chemistry only.
Months of Age													**3[a]**	**6, 9, 12, 15, 21**	**18, 24, (or ET)**	Please see footnote "a" at the bottom of the Appendix 1 table for guidance on scheduling visits for Study Days 44, 60, 72, and 3 Months of Age.
Window						**± 2 days**					**± 7 days**			**± 14 days**	**0–14 days**	The End of Study visit must occur within 0 to 14 days after the date on which the patient reaches 18 or 24 months of age (or ET), depending on the respective cohort. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose. Visits following Day 72 are based on the patient's date of birth.
Milestones (with video)																Reference Study. Videos may be submitted for review by an independent central reviewer. See Sections 11.1, 11.2.1, and 11.3.
CHOP INTEND (with video)	X					X	X	X	X		X		X	X	X	CHOP INTEND will be performed only for patients with 2 copies of *SMN2*. Patients who achieve 3 consecutive CHOP INTEND scores ≥58 will not continue CHOP INTEND assessments. See Sections 11.2.2 and 11.3.
CMAP	X													X	X	To be completed every 6 months, starting at 6 months of age through and including the EOS. .
Demographic/ Medical History	X															See Section 12.1.1.
Physical Exam	X			X	X	X	X	X	X		X		X	X	X	See Section 12.1.2.

Study Period	**Screening**		**Gene Replacement Therapy (In-patient)**			**Follow-up (Outpatient)**									**Clarification**
Study Day	**–30 to –2**	**–14 to –2**	**-1**	**1**	**2**	**7**	**14**	**21**	**30**	**44**	**60**	**72**			**End of Study**	Day 1 assessments will be performed prior to the start of gene replacement therapy infusion. Day 7, 14, 21, 30, 44, 60, and 72 visits are timed based on the Day of AVXS-101 dose (Day 1). Visits on Study Days 44 and 72 are for blood chemistry only.
Months of Age													**3[a]**	**6, 9, 12, 15, 21**	**18, 24, (or ET)**	Please see footnote "a" at the bottom of the Appendix 1 table for guidance on scheduling visits for Study Days 44, 60, 72, and 3 Months of Age.
Window						**± 2 days**					**± 7 days**			**± 14 days**	**0–14 days**	The End of Study visit must occur within 0 to 14 days after the date on which the patient reaches 18 or 24 months of age (or ET), depending on the respective cohort. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose. Visits following Day 72 are based on the patient's date of birth.
Vital Signs/ Weight & Length	(X)		X	(X)*	X	X	X	X	X		X		X	X	X	See Section 12.1.3. Vital signs include blood pressure, respiratory rate, pulse, temperature, and pulse oximetry. (X)*: On Day 1, vital signs, with the exception of blood pressure will be recorded pre-dose and then continuously monitored throughout the gene replacement therapy infusion as follows: Every 15 (± 5) minutes from the start of the infusion for the first 1 hour; Every 30 minutes (± 10 minutes) until 2 hours; Every 2 hours (+ 15 minutes) until 6 hours; Every 4 hours (+ 30 minutes) until 24 hours. Blood pressure will be recorded pre-dose and every 8 hours through 24 hours. Additional monitoring of vital signs will be at the discretion of the Investigator. Temperature will be recorded pre- and post infusion. (X): Screening weight shall be obtained ≤14 days prior to Day 1.

Study Period	**Screening**		**Gene Replacement Therapy (In-patient)**			**Follow-up (Outpatient)**										**Clarification**
Study Day	**–30 to –2**	**–14 to –2**	**-1**	**1**	**2**	**7**	**14**	**21**	**30**	**44**	**60**	**72**			**End of Study**	Day 1 assessments will be performed prior to the start of gene replacement therapy infusion. Day 7, 14, 21, 30, 44, 60, and 72 visits are timed based on the Day of AVXS-101 dose (Day 1). Visits on Study Days 44 and 72 are for blood chemistry only.
Months of Age													**3[a]**	**6, 9, 12, 15, 21**	**18, 24, (or ET)**	Please see footnote "a" at the bottom of the Appendix 1 table for guidance on scheduling visits for Study Days 44, 60, 72, and 3 Months of Age.
Window						**± 2 days**					**± 7 days**			**± 14 days**	**0–14 days**	The End of Study visit must occur within 0 to 14 days after the date on which the patient reaches 18 or 24 months of age (or ET), depending on the respective cohort. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose. Visits following Day 72 are based on the patient's date of birth.
12-Lead ECG	X		X	X	X								X	X	X	A 12-lead ECG, echocardiogram, and 24-hour Holter Monitor are to be completed at Screening, at 3, 6, 9, and 12 months of age, and every 6 months after 12 months of age. For patients not already undergoing 12-lead ECG/24-Hour Holter monitoring/ECHO from 3 months of age, these tests will be performed when patients are initially consented for them (ICF corresponding to protocol version 3.0 or later), and then according to the Schedule of Assessments. Note that 12-lead ECG and 24-hour Holter monitor are also performed during Days -1, 1, and 2. See Section 12.1.4, 12.1.5, and 12.1.6 for more details.
Echocardiogram	X												X	X	X	
24-Hour Holter Monitor	X		X	X	X								X	X	X	
Pulmonary Examination	X		X		X	X	X	X	X		X		X	X	X	See Section 12.1.7.
Swallowing Test	X													X	X	Completed every 6 months, starting at 6 months of age through and including the EOS vist. Each test should include consistency tested. See Section 12.1.8.

Study Period	**Screening**		**Gene Replacement Therapy (In-patient)**			**Follow-up (Outpatient)**									**Clarification**
Study Day	**–30 to –2**	**–14 to –2**	**-1**	**1**	**2**	**7**	**14**	**21**	**30**	**44**	**60**	**72**			**End of Study**	Day 1 assessments will be performed prior to the start of gene replacement therapy infusion. Day 7, 14, 21, 30, 44, 60, and 72 visits are timed based on the Day of AVXS-101 dose (Day 1). Visits on Study Days 44 and 72 are for blood chemistry only.
Months of Age													**3[a]**	**6, 9, 12, 15, 21**	**18, 24, (or ET)**	Please see footnote "a" at the bottom of the Appendix 1 table for guidance on scheduling visits for Study Days 44, 60, 72, and 3 Months of Age.
Window						**± 2 days**					**± 7 days**			**± 14 days**	**0–14 days**	The End of Study visit must occur within 0 to 14 days after the date on which the patient reaches 18 or 24 months of age (or ET), depending on the respective cohort. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose. Visits following Day 72 are based on the patient's date of birth.
Photographs of Infusion Site				(X)	X	X	X	X	X							(X): Day 1 photograph of infusion site should be performed after infusion. See Section 12.1.9.
Hematology/ Chemistry/ Urinalysis		X			(X)	X	(X)[#]	X	(X)*	(X)[#]	X	(X)[#]	X	X	X	(X): Laboratory samples collected on Day 2 to be processed locally. (X)*: Chemisry and urinalysis only. No hematology (X)[#]: Chemistry only. See Sections 0, 12.1.10.2, and 12.1.10.3.
CK-MB or Troponin I		X				X			X					X	X	CK-MB or troponin-I will be collected at Screening, at the Day 7 and Day 30 visits, and every three months starting at the 6 months of age visit through and including the EOS visit. Patients screened and dosed before IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 will be tested for CK-MB throughout the study. Patients screened before IRB/EC approval of Protocol version 2.0 incorporating Amendment 1 but dosed afterwards, will be tested for CK-MB at screening but for Troponin I at baseline and all subsequent visits. Patients screened after IRB/EC

Study Period	**Screening**		**Gene Replacement Therapy (In-patient)**			**Follow-up (Outpatient)**									**Clarification**
Study Day	**–30 to –2**	**–14 to –2**	**-1**	**1**	**2**	**7**	**14**	**21**	**30**	**44**	**60**	**72**			**End of Study**	Day 1 assessments will be performed prior to the start of gene replacement therapy infusion. Day 7, 14, 21, 30, 44, 60, and 72 visits are timed based on the Day of AVXS-101 dose (Day 1). Visits on Study Days 44 and 72 are for blood chemistry only.
Months of Age													**3[a]**	**6, 9, 12, 15, 21**	**18, 24, (or ET)**	Please see footnote "a" at the bottom of the Appendix 1 table for guidance on scheduling visits for Study Days 44, 60, 72, and 3 Months of Age.
Window						**± 2 days**					**± 7 days**			**± 14 days**	**0–14 days**	The End of Study visit must occur within 0 to 14 days after the date on which the patient reaches 18 or 24 months of age (or ET), depending on the respective cohort. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose. Visits following Day 72 are based on the patient's date of birth.
																approval of Protocol version 2.0 incorporating Amendment 1 will be tested for Troponin 1 from screening onwards. The pre-and post-dose cardiac laboratory analytes must match. See Secton 12.1.10.2.
Biological Mother Virus/Serology Testing	X															See Secton 12.1.10.4.
Anti-AAV9 Ab Screen in Biological Mother	X															Patient samples for anti-AAV9 screening must be collected if biological mother's titer result is >1:50. See Secton 12.1.10.6.
Genetic Re-confirmation Testing	X															See Secton 12.1.10.5.
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	See Section 13

Study Period	Screening		Gene Replacement Therapy (In-patient)			Follow-up (Outpatient)										Clarification
Study Day	**–30 to –2**	**–14 to –2**	**-1**	**1**	**2**	**7**	**14**	**21**	**30**	**44**	**60**	**72**			**End of Study**	Day 1 assessments will be performed prior to the start of gene replacement therapy infusion. Day 7, 14, 21, 30, 44, 60, and 72 visits are timed based on the Day of AVXS-101 dose (Day 1). Visits on Study Days 44 and 72 are for blood chemistry only.
Months of Age													**3[a]**	**6, 9, 12, 15, 21**	**18, 24, (or ET)**	Please see footnote "a" at the bottom of the Appendix 1 table for guidance on scheduling visits for Study Days 44, 60, 72, and 3 Months of Age.
Window						**± 2 days**					**± 7 days**			**± 14 days**	**0–14 days**	The End of Study visit must occur within 0 to 14 days after the date on which the patient reaches 18 or 24 months of age (or ET), depending on the respective cohort. Visits prior to and including the Day 72 visit are timed based on the Day of AVXS-101 dose. Visits following Day 72 are based on the patient's date of birth.
Prior and Concomitant Medications	Collected from 2 Weeks before gene replacement therapy until End of Study visit															See Secion 9.2.2.

Note: Missed visits should be rescheduled as soon as possible, but within 7 days.
Ab = antibody; BSIDv03 = Bayley Scales of Infant and Toddler Development, version 3; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CMAP = compound motor action potential; ECG = electrocardiogram; EOS = End of Study; ET = early termination; ICF = informed consent form; SAE = serious adverse event; WHO = World Health Organization

[a.] The 3 months of age visit (also known as the Day 90 visit) is based on age (90 days=3 months of age) and all assessments at this visit may coincide with Day 44, Day 60 or Day 72. If visit windows for these visits overlap, study sites should schedule the visits as far apart as possible within the respective visit windows (i.e. schedule the earlier visit in the earlier part of its' window and schedule the later visit in the latter part of the window). In instances where this is not possible, it is acceptable for an overlapping visit to occur as long as all assessments between visits are performed. In such instances, the lab requisition form should be marked for all visits that apply and [redacted] must be notified. At combined visits, one of each assessment scheduled to occur in both visits must be completed. If the visit is a combination of Day 44 or Day 72 and Day 90, then the visit will be conducted as Day 90. If the visit is a combination of Day 44 or Day 72 and Day 60, then the visit will be conducted as Day 60. If the visit combines Day 60 and Day 90, with or without Days 44 and/or 72, then the visit will be conducted as Day 60. **Sites are advised to collect chemistry samples at each visit during the prednisolone administration period and any visits up to two weeks after last dose of prednisolone. If visits are combined as described above, then the site investigator should make every effort to monitor chemistry at the appropriate time points using unscheduled visits if necessary.**

APPENDIX 2. POST-MORTEM PLAN

Post-mortem tissue/organ collection and autopsy, where possible, will be requested for any patient who receives treatment with AVXS-101 and subsequently expires, at any timepoint. The tissue and organ collection will be performed by a contracted vendor or hospital pathologist who will deploy a pathologist and pathology assistant, as necessary, to the funeral home of the deceased or other appropriate location to perform the autopsy and tissue collection.

During the procedure, multiple tissues along with the entire spinal cord will be collected for research purposes, including up to 7 sections or pieces from each organ and each region of the spinal cord. Upon collection, these tissue samples will be provided to AveXis for analysis. Tissue analysis will be done to determine whether the vector transduced the expected motor neurons and if the SMN gene was expressed. These results will demonstrate whether the vector delivered the therapeutic gene as expected. Tissue samples collected will also be available for histology and immunohistochemistry, allowing the state of the motor neurons and muscles to be examined.

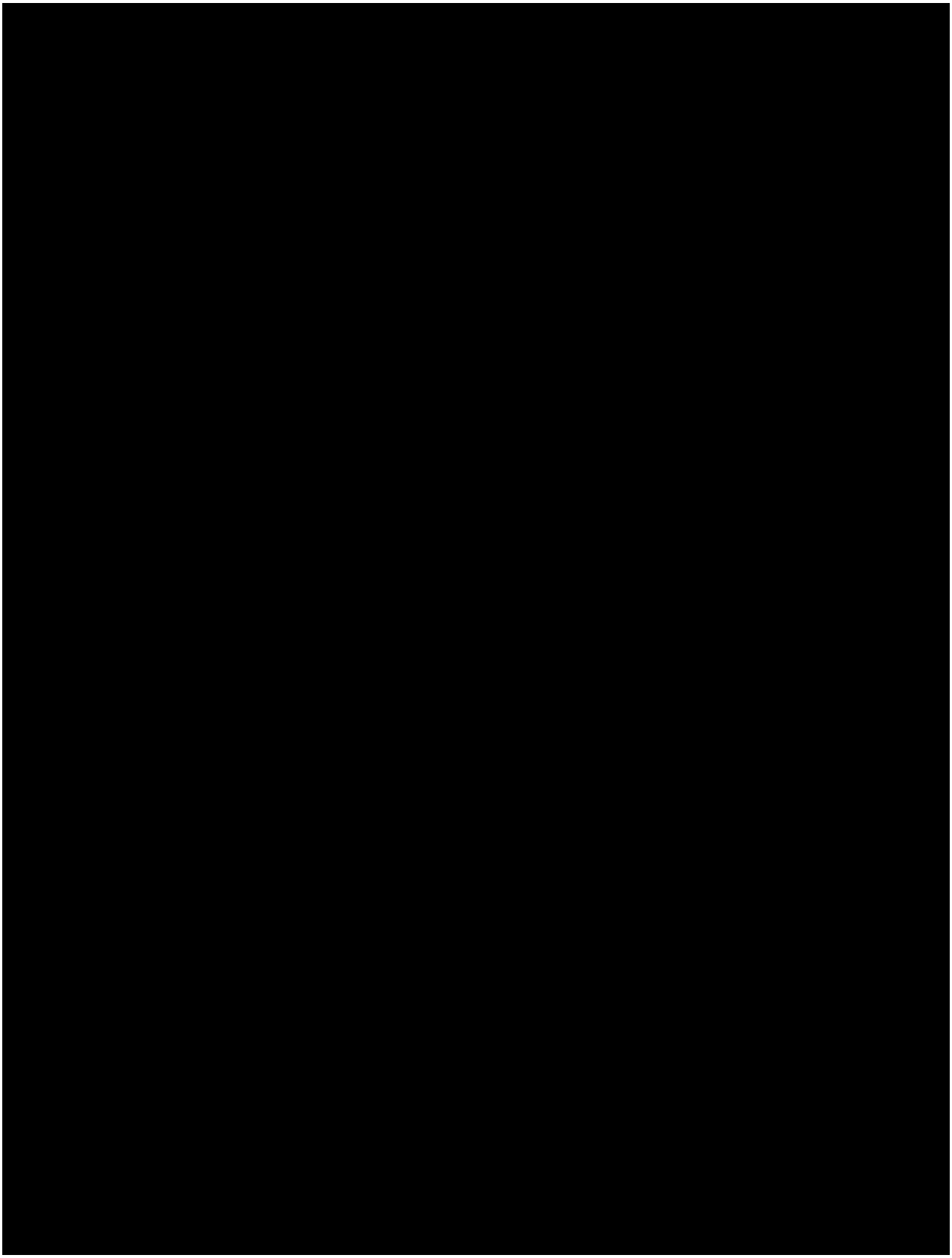
Specifically, tissue samples from the spinal cord, muscles, and organs will be collected as indicated in Table 6. Tissue samples will be frozen or fixed (e.g., 2% paraformaldehyde) for appropriate analysis.

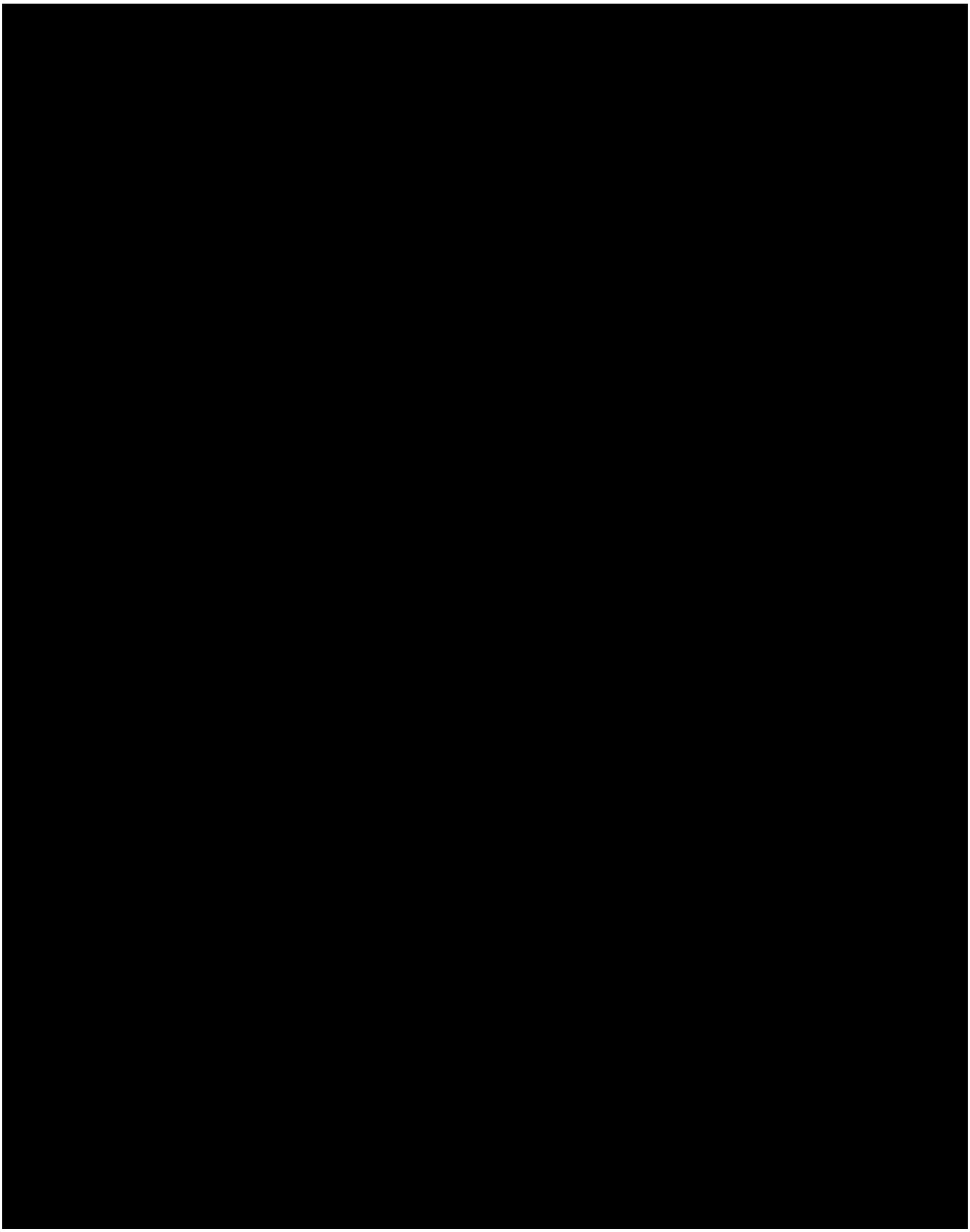
Families will be asked to consent to authorization for tissue and organ collection and prior to any sign of moribund or death by the clinical team conducting the study. Declining the post-mortem tissue/organ collection will not prevent patients from participating in the study.

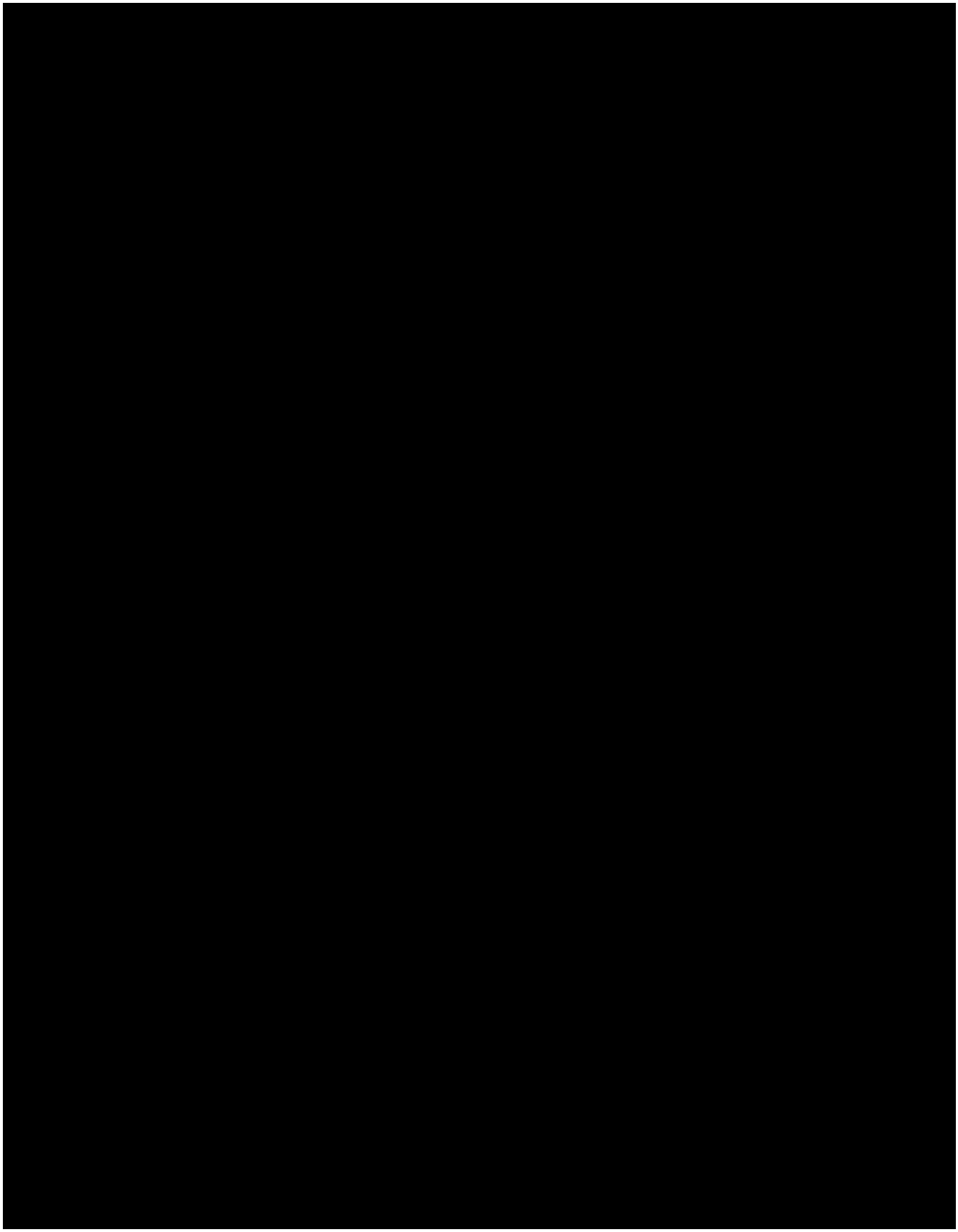
Table 6 - Tissue Sample for Analysis

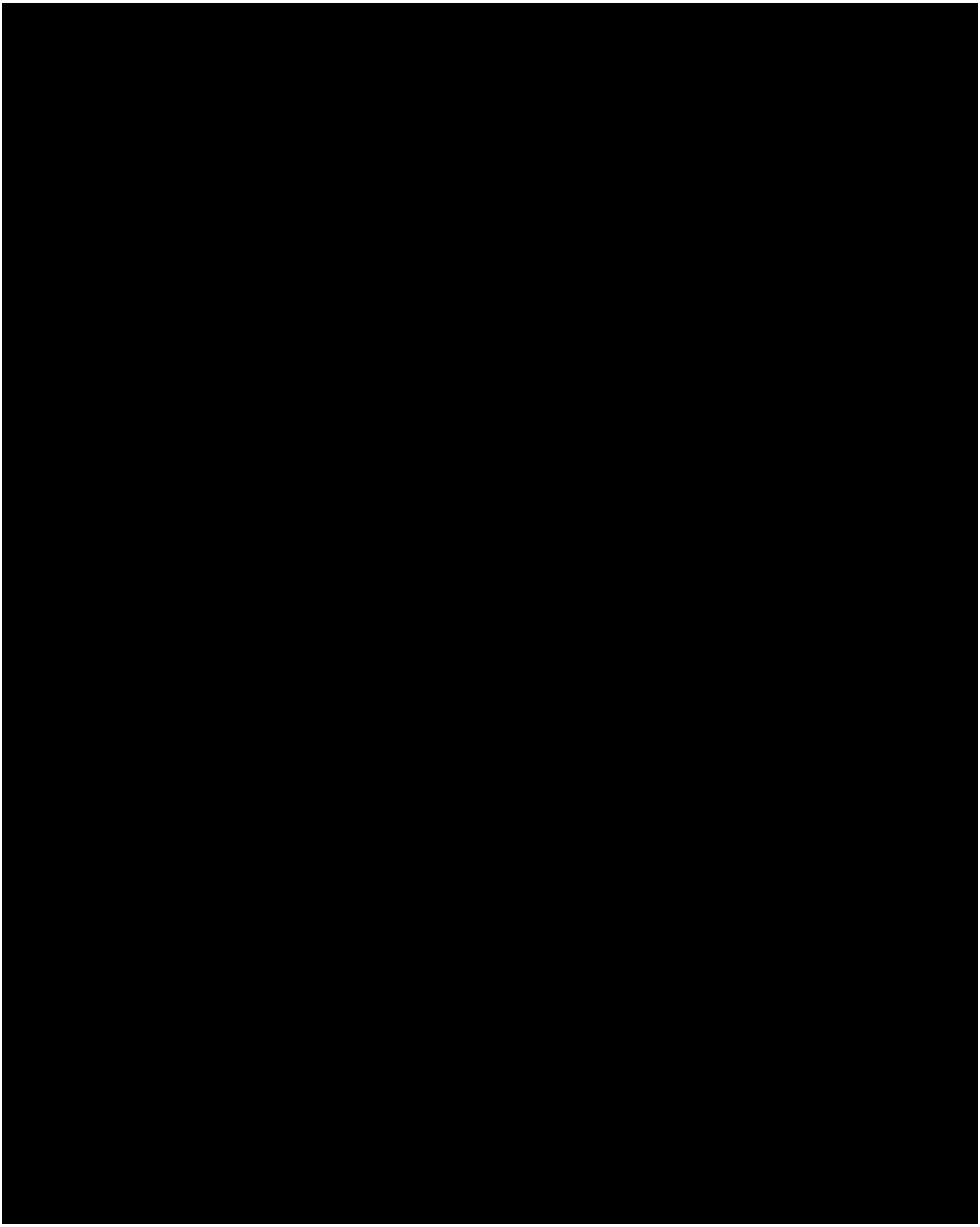
Brain	Spinal Cord	Muscles	Organs
Motor cortex	Cervical spinal cord	Diaphragm	Spleen
Layer 5 motor cortex	Thoracic spinal cord	#6/#7 Rib with intercostal muscle and nerve	Kidney
Brain stem	Lumbar spinal cord	Psoas muscle	Small intestine
	Sacral spinal cord		Large intestine
	Dorsal root		Pancreas
	Cervical level		Stomach
	Ventral root		Lung
	Cervical level		Heart
	DRG root		Liver
	Cervical level		Inguinal lymph node
	Cerebrospinal fluid		Gonads

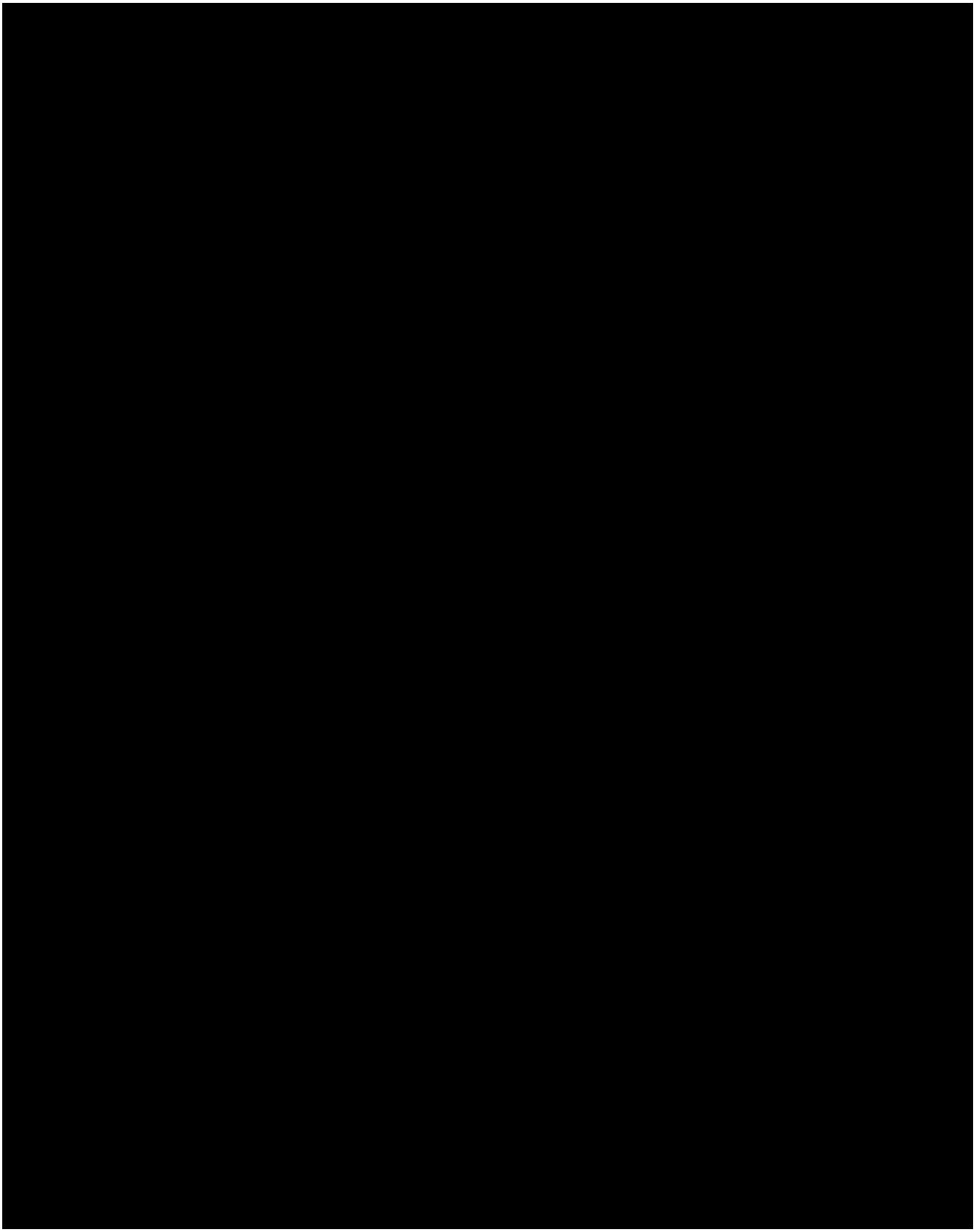
APPENDIX 3. BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT (VERSION 3)

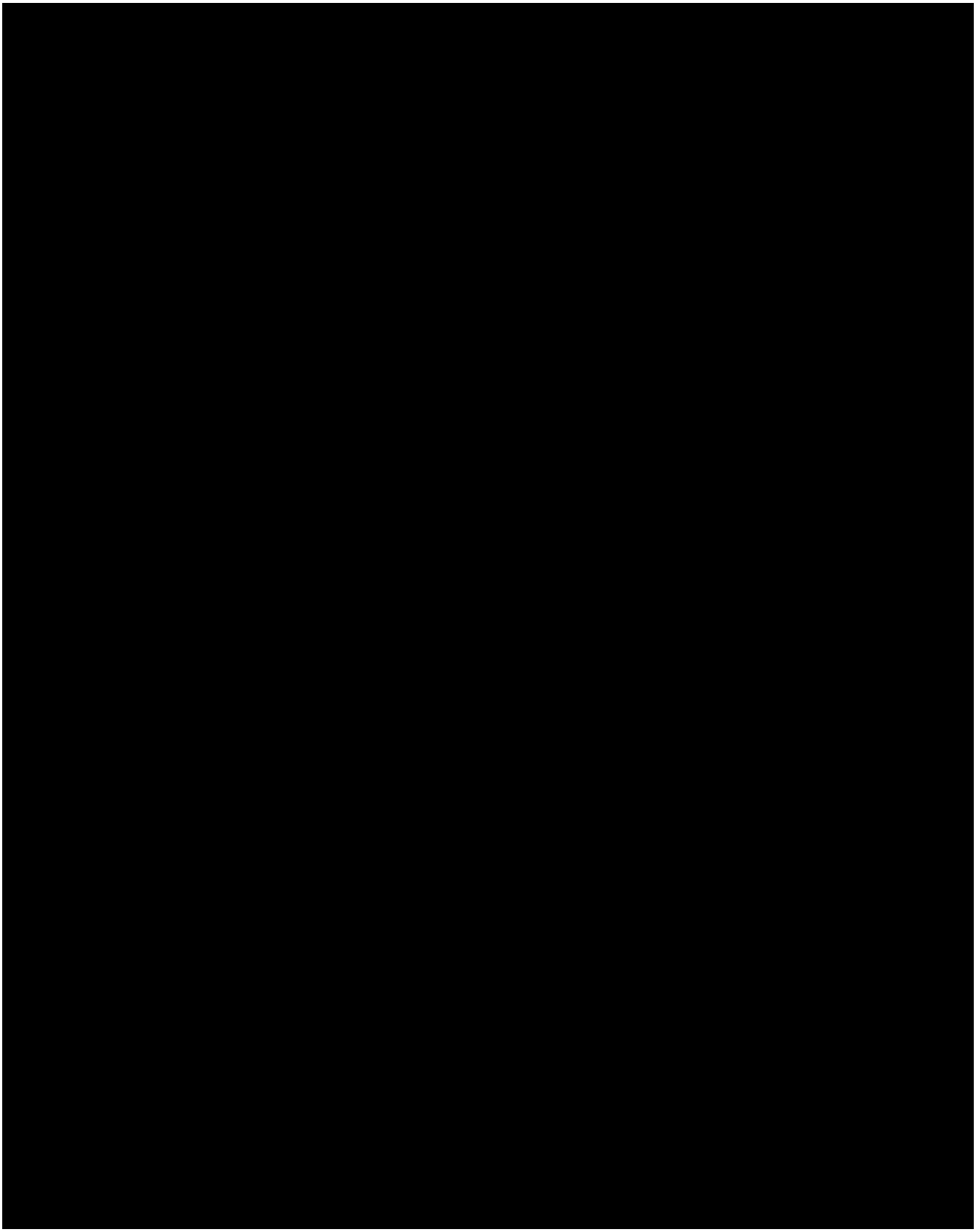


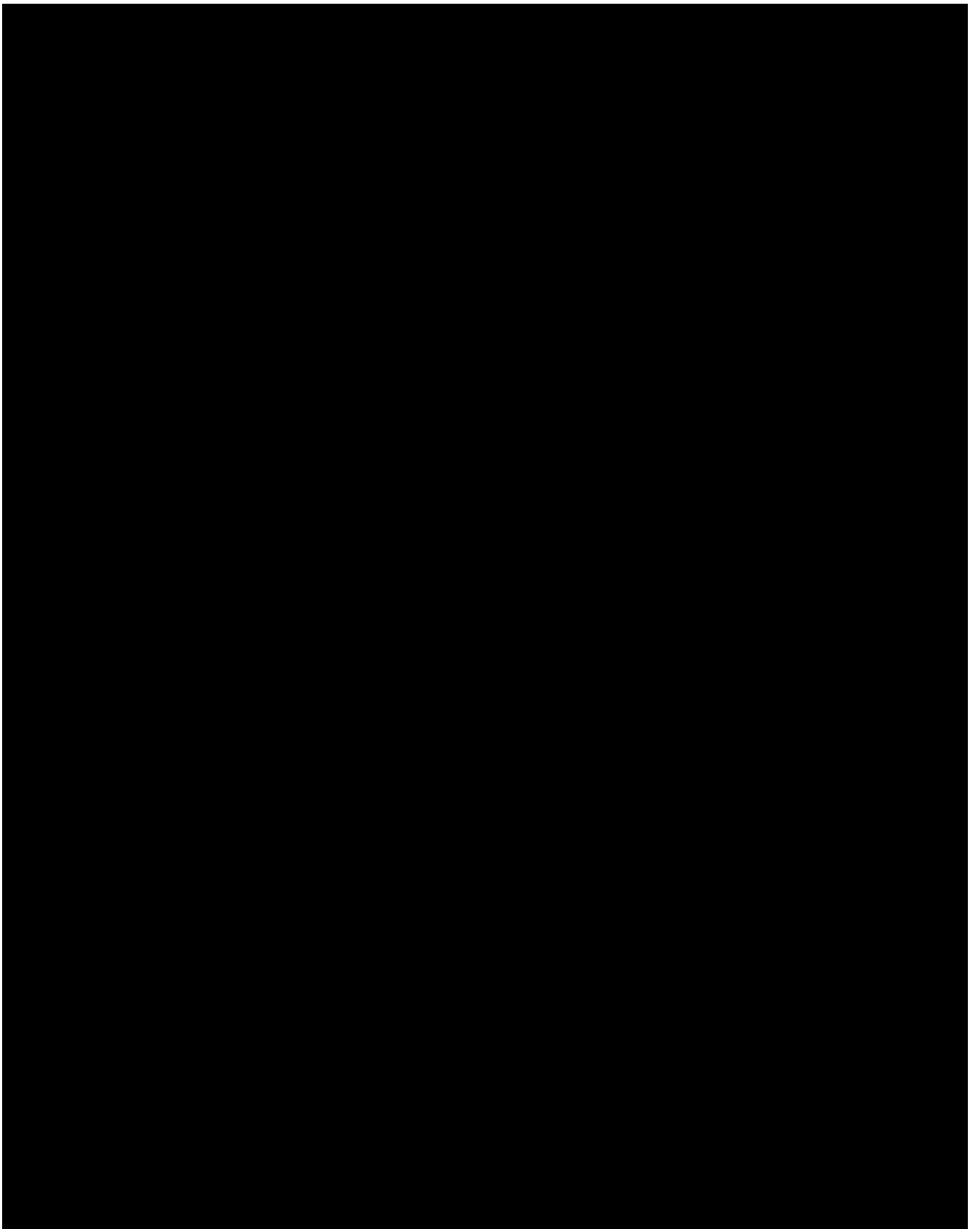


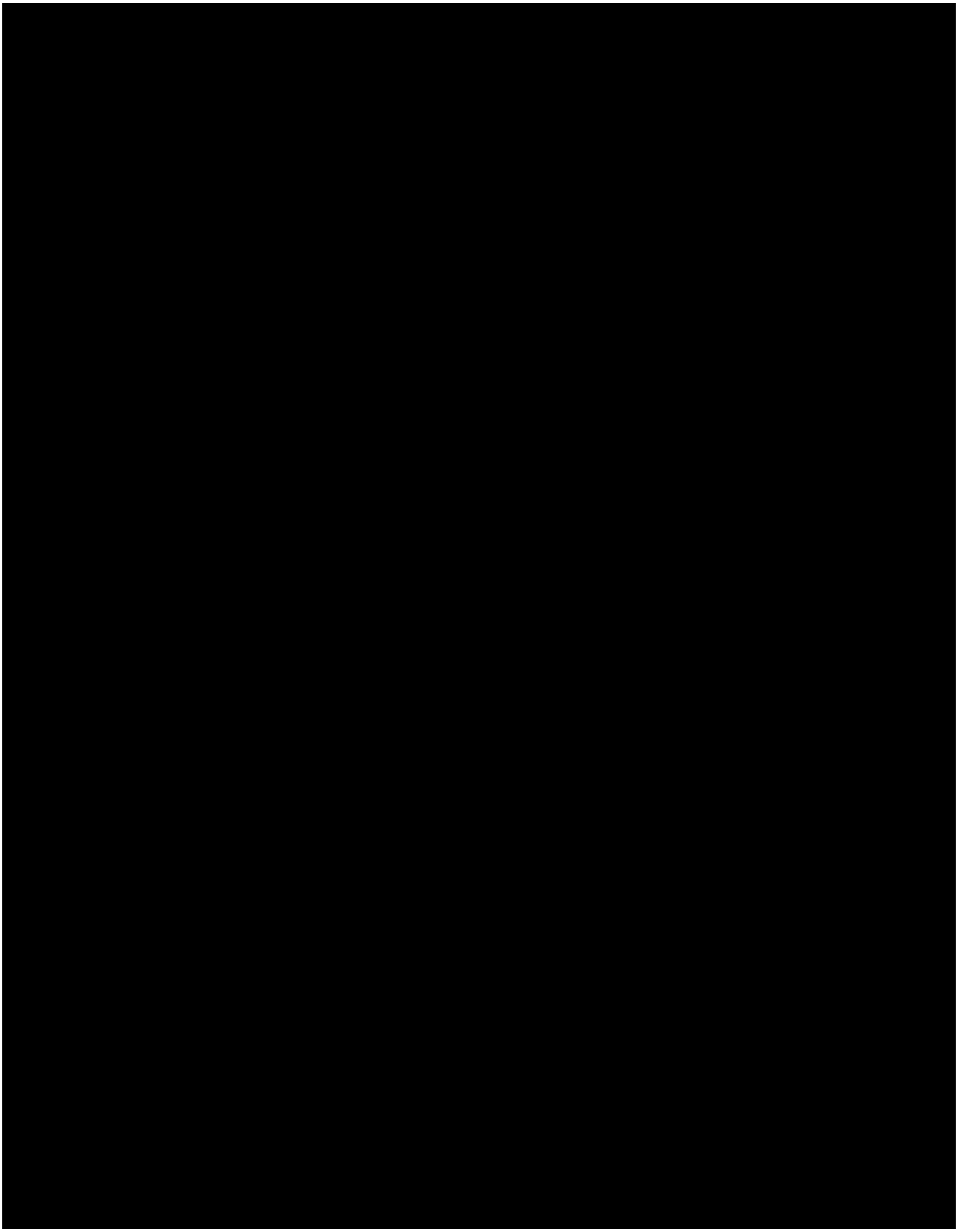


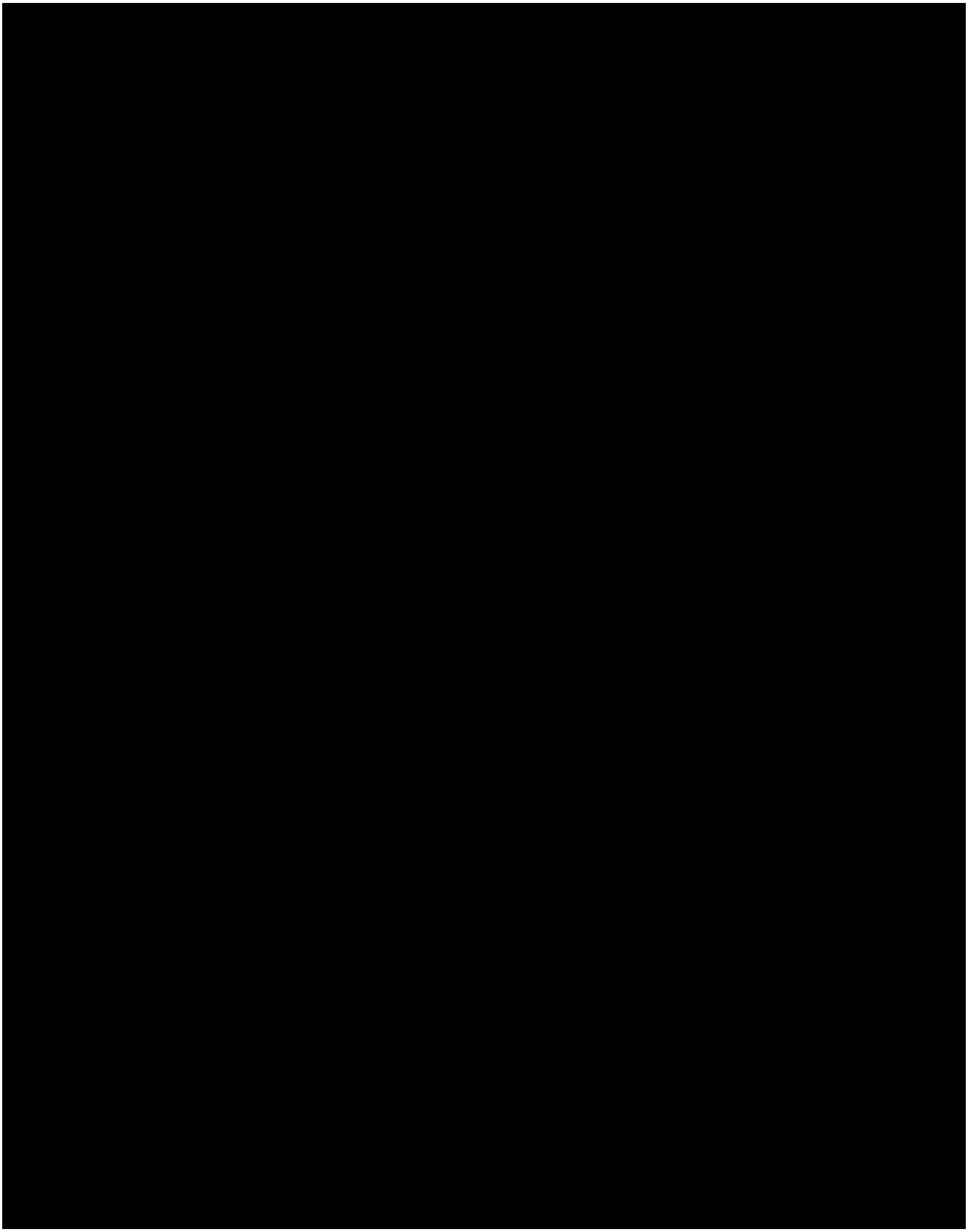


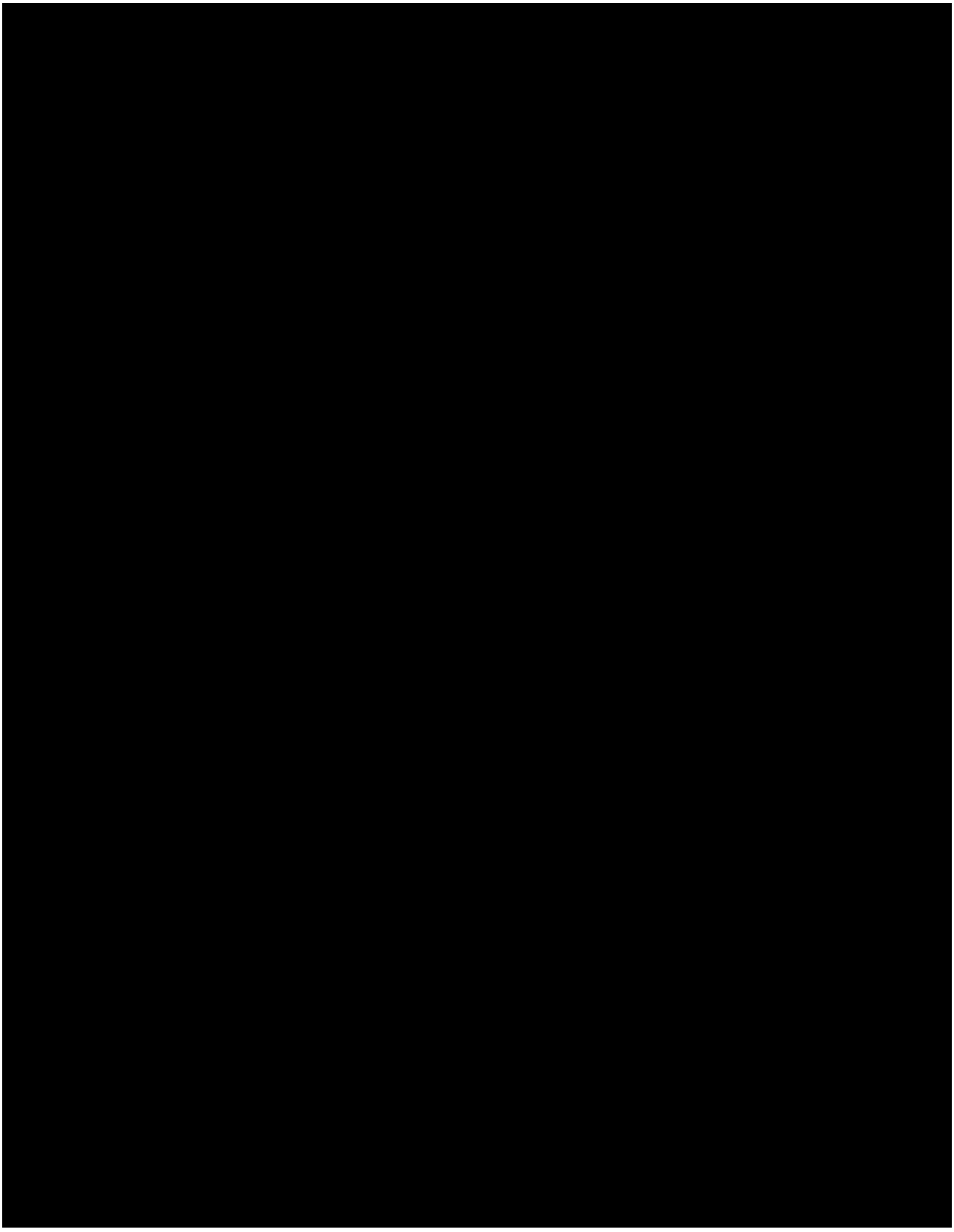


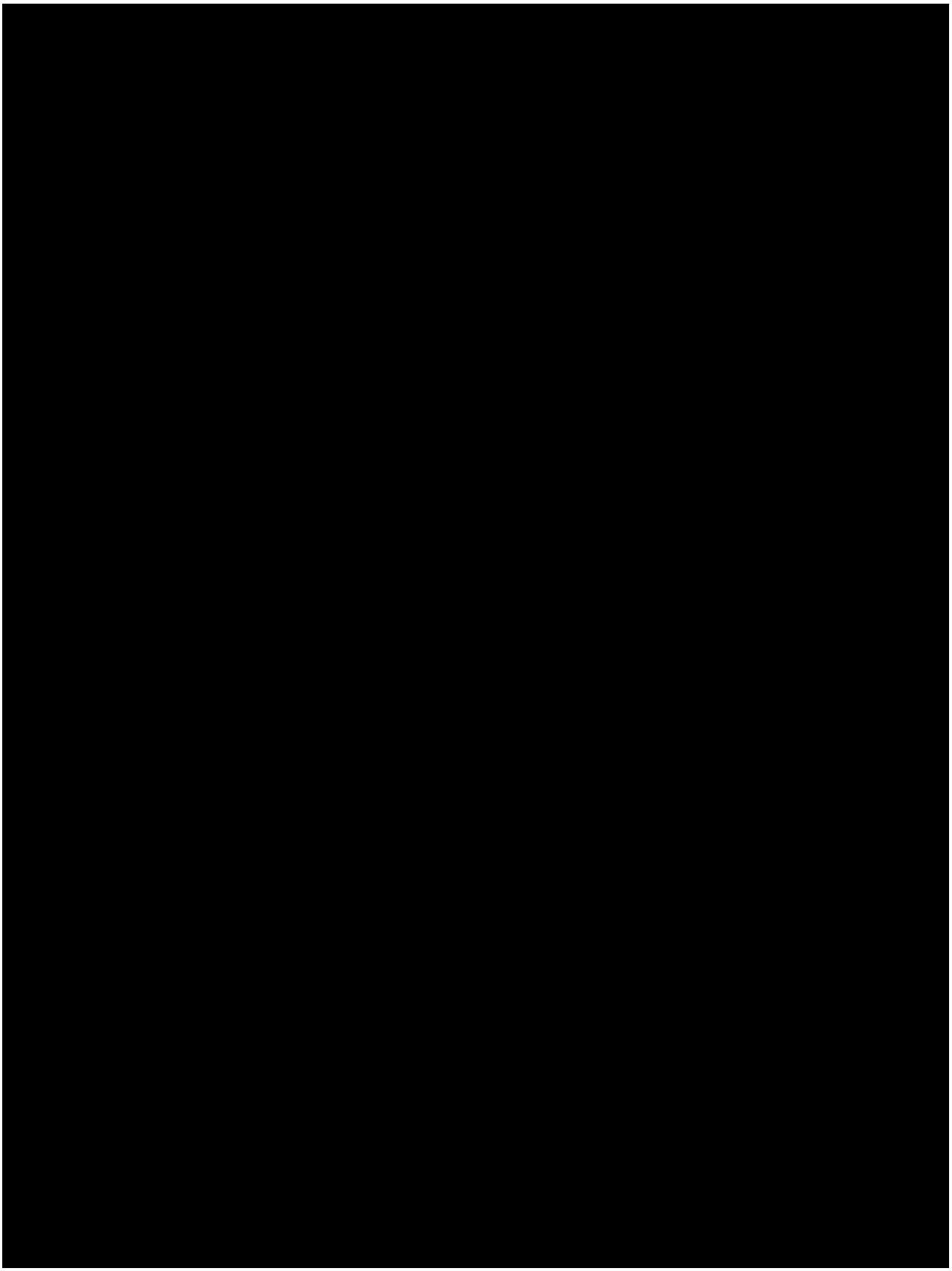


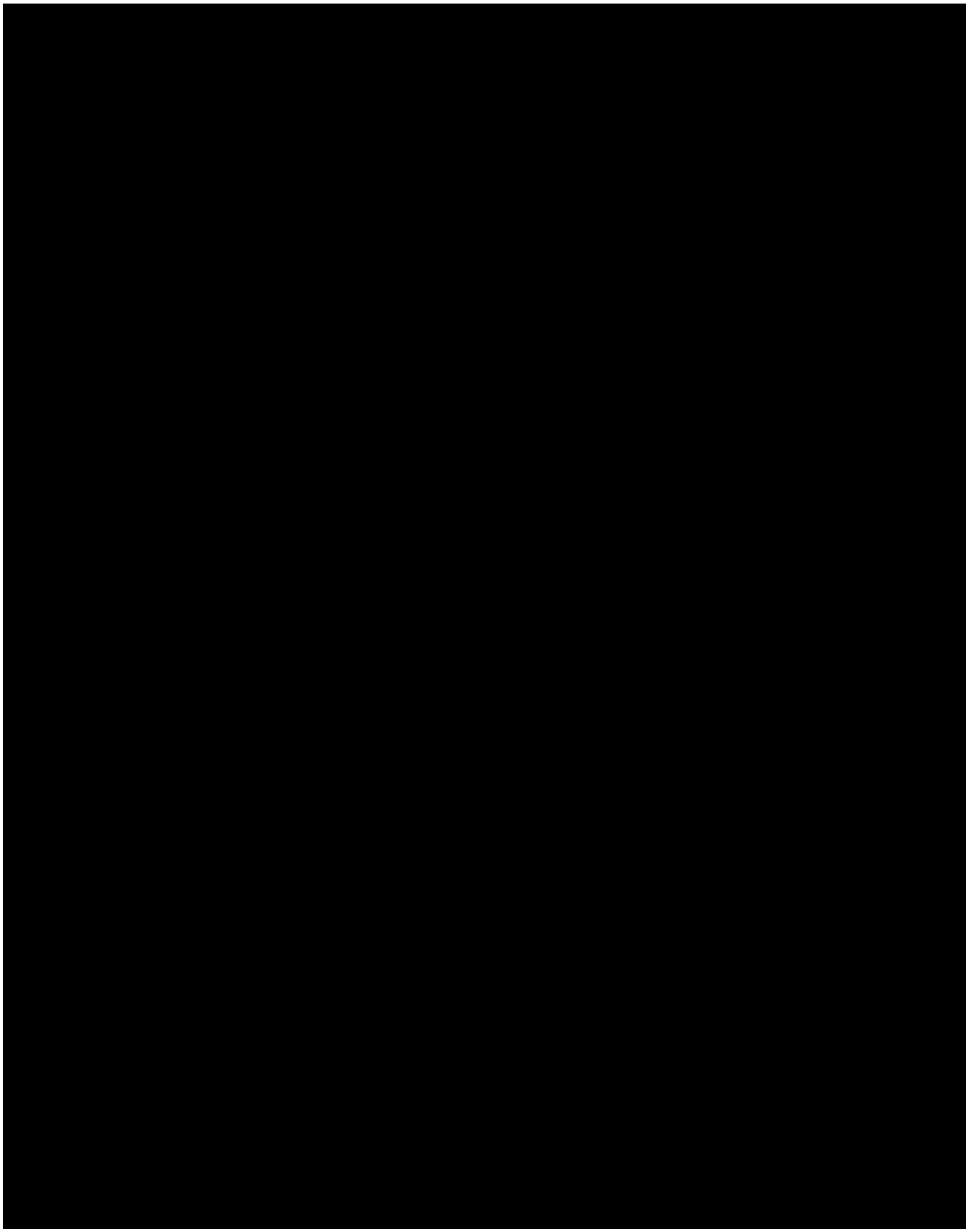


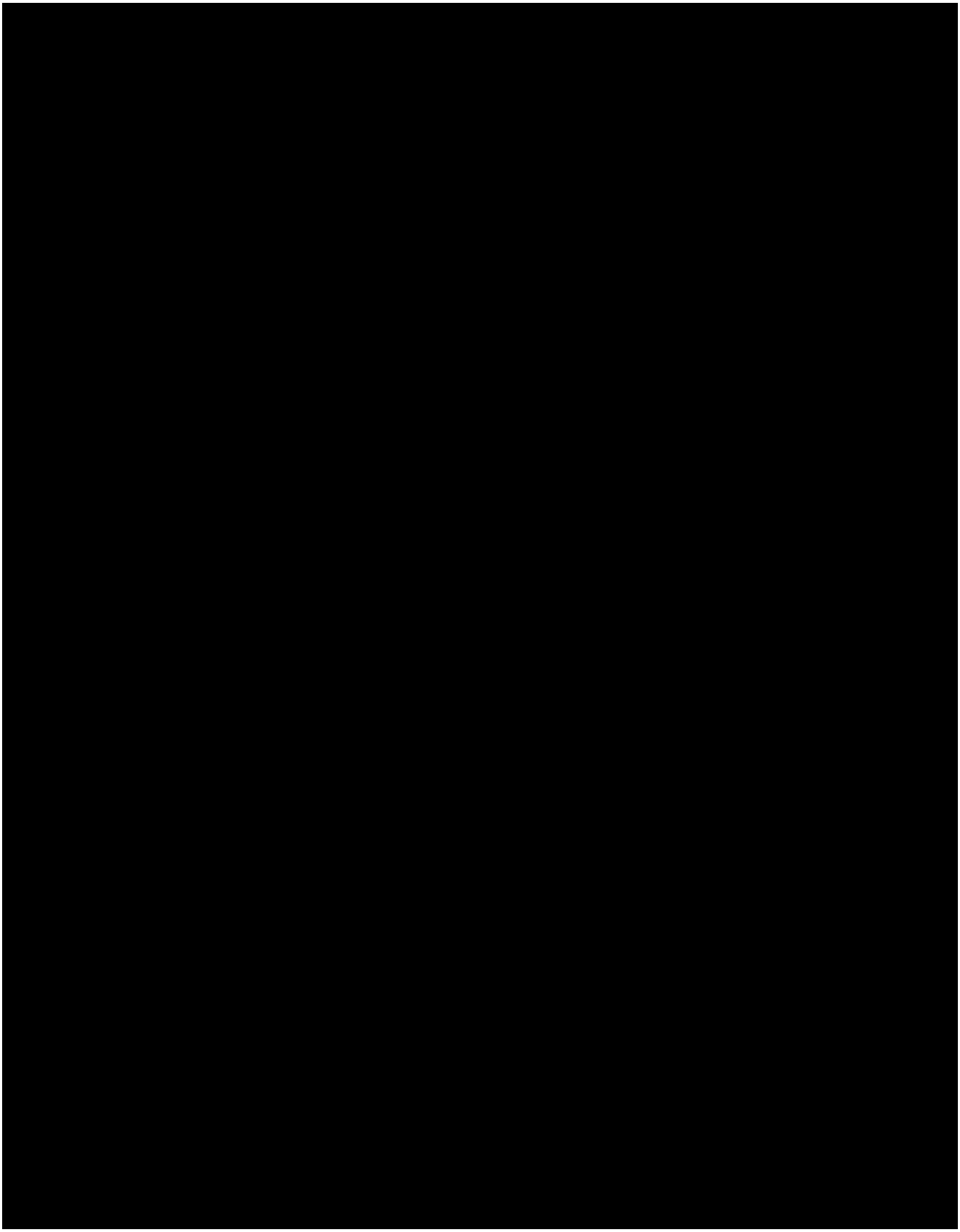


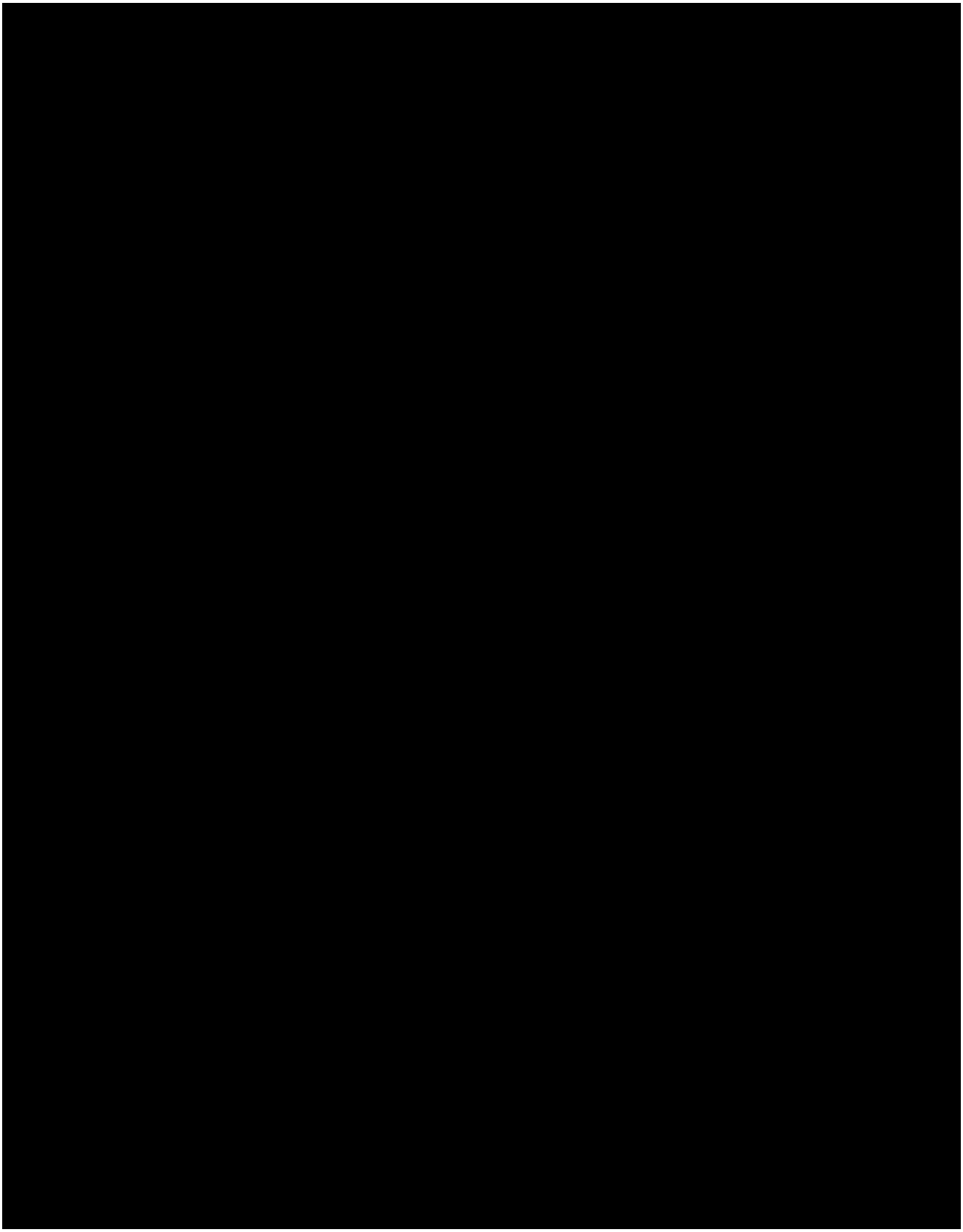


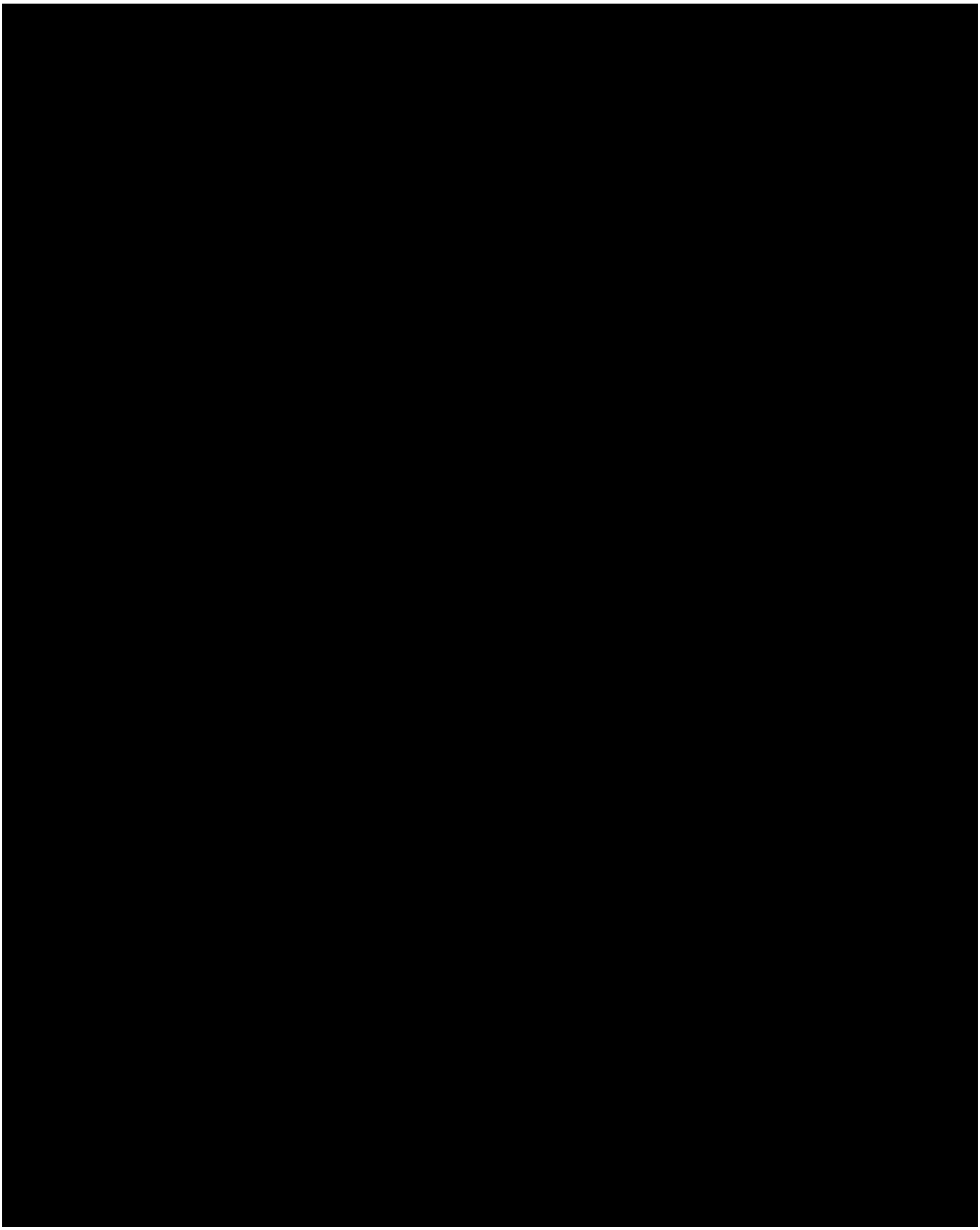


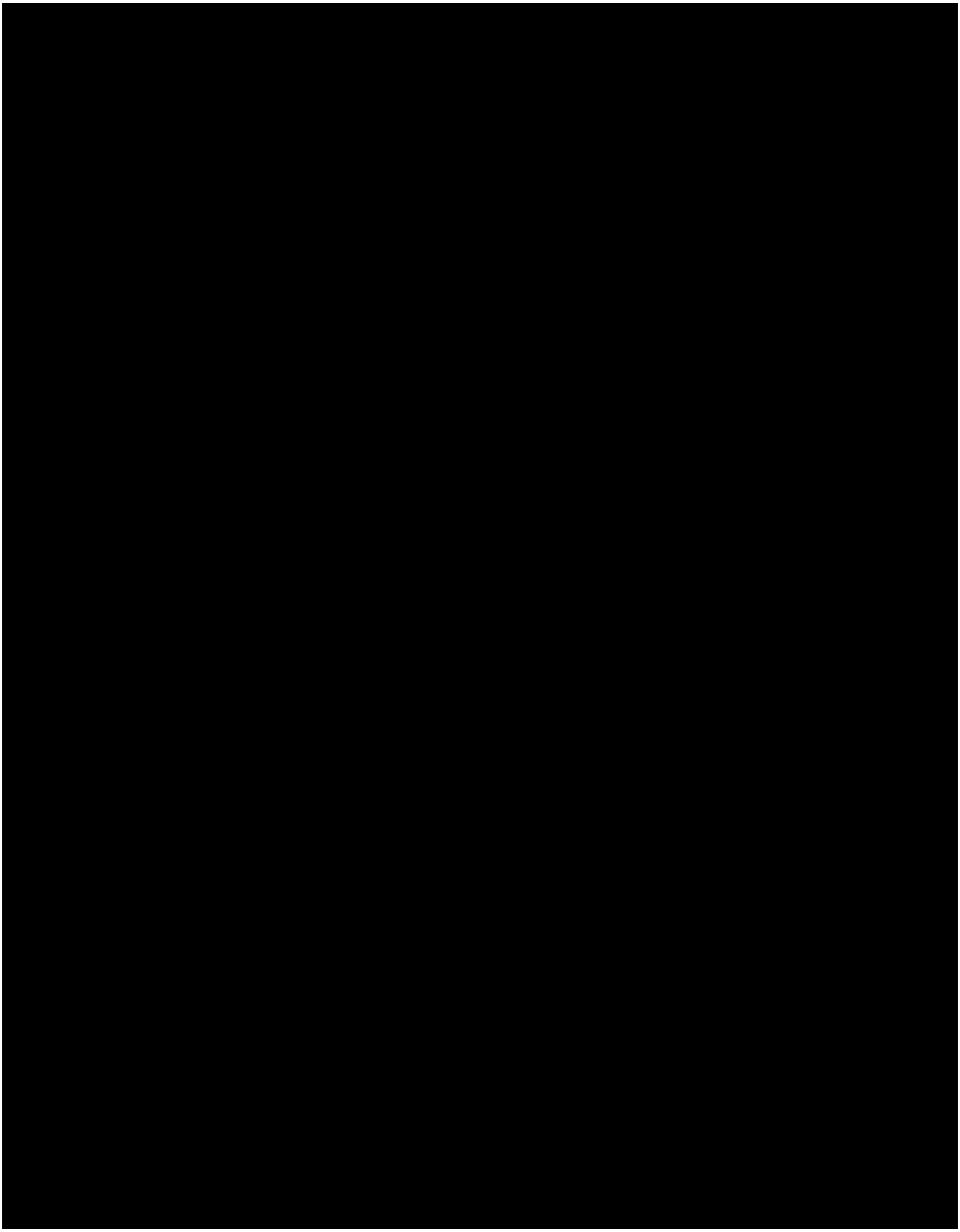












APPENDIX 4. PERFORMANCE CRITERIA FOR BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT (VERSION 3) DEVELOPMENTAL MILESTONES

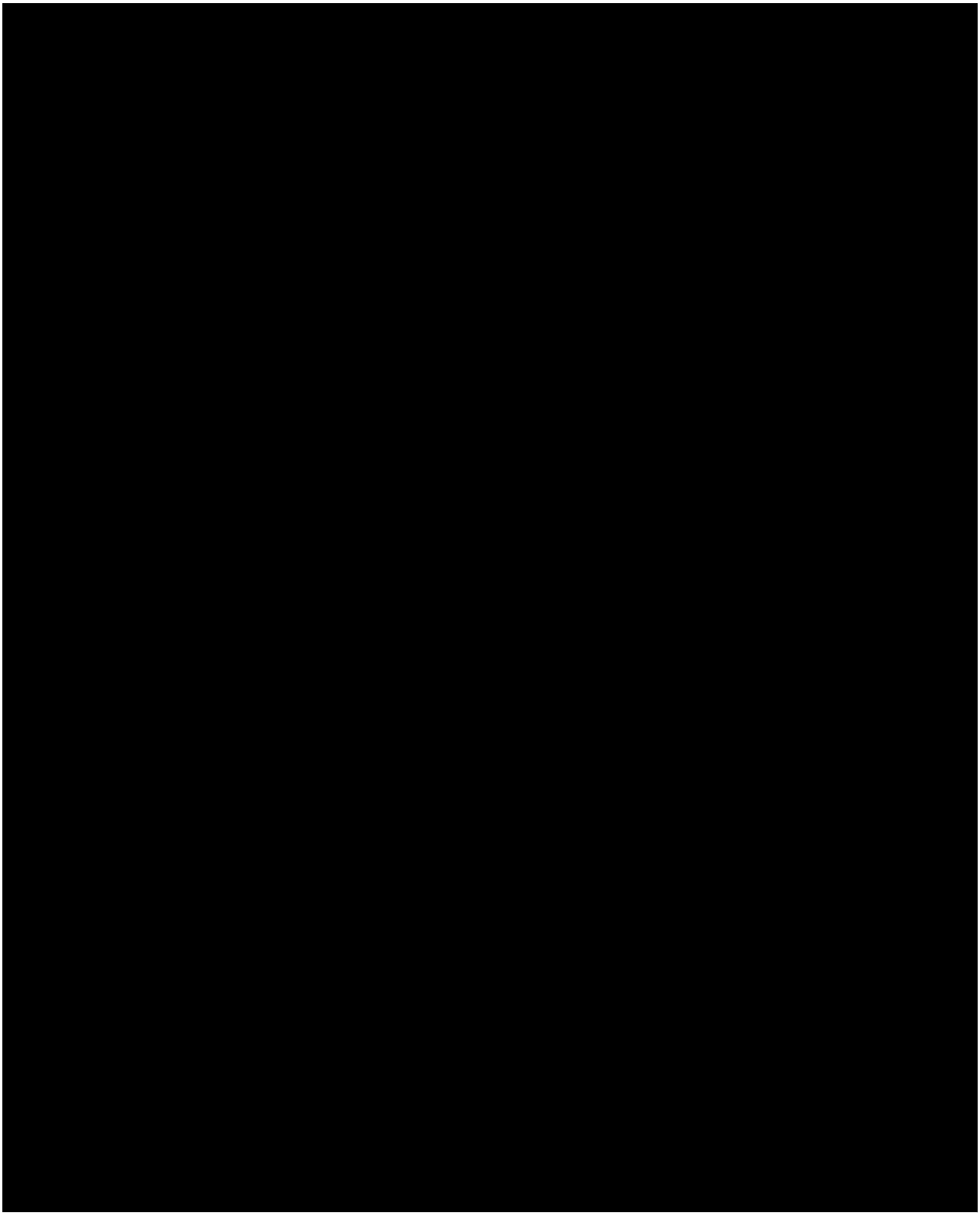
Developmental Milestone	Performance Criteria
Head Control – Gross Motor Subtest Item #4	[redacted]
Rolls from Back to Sides – Gross Motor Subtest Item #20	
Sits Without Support – Gross Motor Subtest Item #26	
Stands With Assistance - Gross Motor Subtest Item #33	
Crawls – Gross Motor Subtest Item #34	
Pulls to Stand – Gross Motor Subtest Item #35	
Walks With Assistance – Gross Motor Subtest Item #37	
Stands Alone – Gross Motor Subtest Item #40	
Walks Alone – Gross Motor Subtest Item #43	

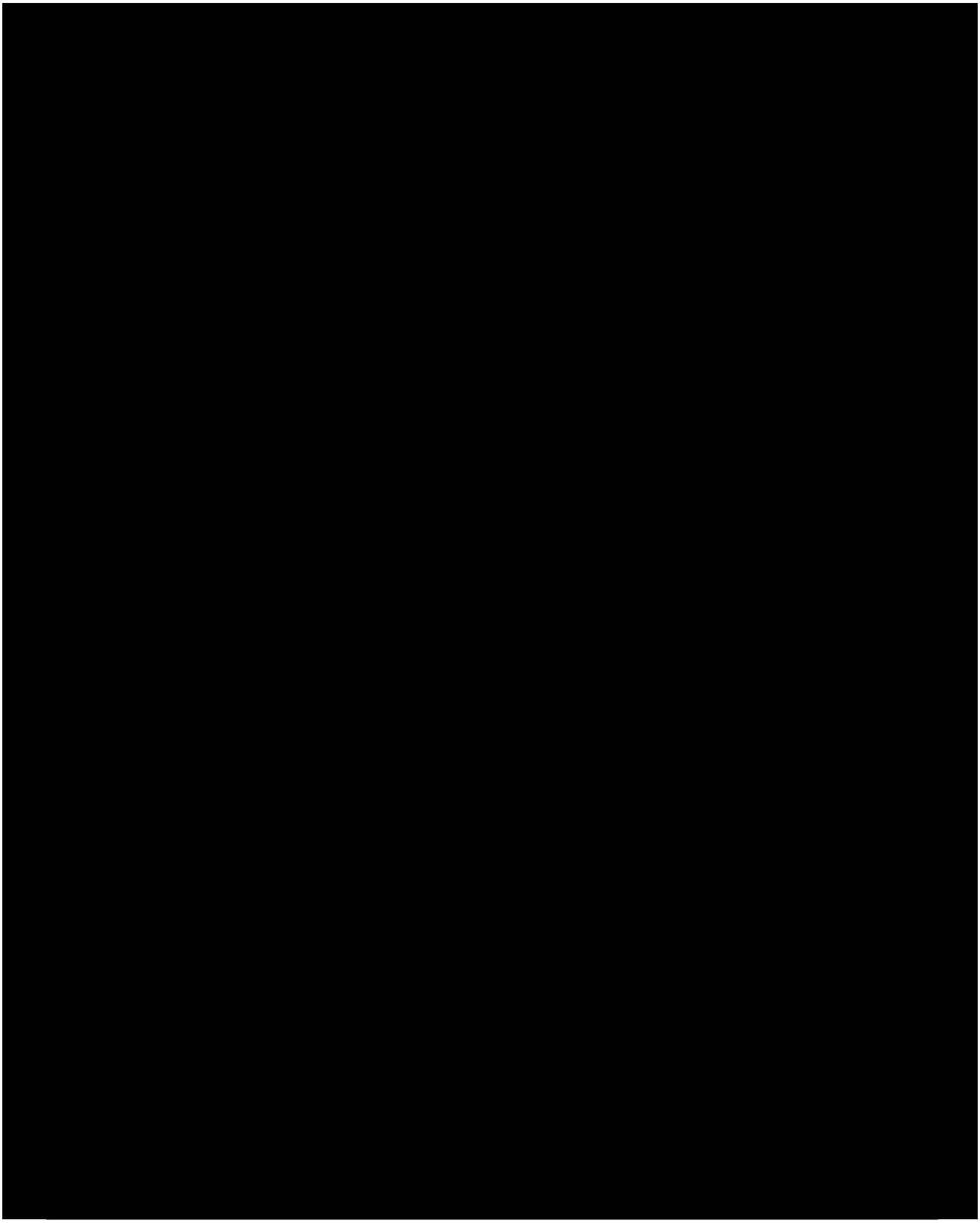
APPENDIX 5. PERFORMANCE CRITERIA FOR WORLD HEALTH ORGANIZATION (WHO) DEVELOPMENT MILESTONES

Gross Motor Milestone	Performance Criteria
Sitting without support	[redacted]
Hands-and-knees crawling	
Standing with assistance	
Walking with assistance	
Standing alone	
Walking alone	

WHO = World Health Organization
Source: World Health Organization Multicentre Growth Reference Trial Group [22]

APPENDIX 6. CHOP INTEND





APPENDIX 7. 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

1. 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.”
2. 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.
3. Medical progress is based on research that ultimately must include studies involving Human subjects.
4. 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.
5. Medical research is subject to ethical standards that promote and ensure respect for all Human subjects and protect their health and rights.
6. 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 patients.

7. 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 patients. The responsibility for the protection of research patients must always rest with the physician or other health care professionals and never with the research patients, even though they have given consent.
8. 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 patients set forth in this Declaration.
9. Medical research should be conducted in a manner that minimizes possible harm to the environment.
10. 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.
11. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
12. 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 patients.
13. Appropriate compensation and treatment for patients who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

1. 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 patients.
2. 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.
3. 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

1. 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.

2. 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

1. 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.

2. 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 patients and information regarding provisions for treating and/or compensating patients 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

1. 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 patients 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 serious adverse events. 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

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

Informed Consent

1. Participation by individuals capable of giving informed consent as patients 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.
2. 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 patients 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 patients should be given the option of being informed about the general outcome and results of the study.
3. 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.
4. 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.
5. 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.
6. Research involving patients 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 patients 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.

7. 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.
8. 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

1. 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

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

Research Registration and Publication and Dissemination of Results

1. Every research study involving Human subjects must be registered in a publicly accessible database before recruitment of the first subject.
2. 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

1. 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 8. SUMMARY OF CHANGES

See next page.

AVXS-101

AVXS-101-CL-304

Protocol Title:	A Global Study of a Single, One-Time Dose of AVXS-101 Delivered to Infants with Genetically Diagnosed and Pre-symptomatic Spinal Muscular Atrophy with Multiple Copies of *SMN2*
Summary of Changes:	Protocol Version 6.0, Amendment 5, 28 July 2020

SUMMARY AND JUSTIFICATION OF CHANGES

This protocol amendment was required in order to:

- address COVID-19 related impact on trial conduct
- apply consistent definitions of the primary endpoint and exploratory endpoints
- clarify the collection and central review of videos of efficacy parameters
- update the schedule of assessments to align protocol changes for consistency
- address specific requests from Competent Authorities regarding Safety Reporting timelines and Hy's Law Criteria
- remove text that is not applicable for sample size calculation
- make administrative changes
- make minor changes and edits for error correction, clarification, and overall consistency.

The following changes were made to the Protocol from Version 5.0 Amendment 4 dated 26 Nov 2019 to Version 6.0 Amendment 5 dated 28 Jul 2020.

SUMMARY OF CHANGES:

The section below describes the changes incorporated into this version of the protocol.

Protocol Version 6.0 Incorporating Amendment 5 includes the following changes

Description of Change	Location(s)	Rationale for Change
Replaced [redacted]	Signature page	To update per company administrative changes
Updated the list of abbreviations by adding ADL, AT, BSIDv03, and TBL	Section 4: List of Abbreviations and Definitions of Terms	To align changes in the protocol
Text added to allow changes in study conduct during the COVID-19 pandemic (or other similar catastrophic event): - change to the conduct of study visits - when a patient may be considered withdrawn due these factors, versus withdrawn consent - change to the manner in which efficacy and safety assessments are performed - change to the manner in which monitoring visits are performed - the process of obtaining remote informed consent	Section 7.1: Overall Trial Design Section 8.3: Patient Withdrawal Criteria Section 11: Assessment of Efficacy Section 12: Assessment of Safety Section 16.1: Study Monitoring Section 18.3: Written Informed Consent	To address the impact of the COVID-19 pandemic (or other similar catastrophic event) on: - a site's ability to conduct on-site study visits and efficacy and safety assessments per protocol - a subject's willingness or ability to continue participating in the study - a monitor's ability to perform on-site monitoring visits - a site's ability to follow the usual process of obtaining written informed consent
References to developmental milestones were added to further define the primary/secondary objectives	Synopsis: Objectives Sections 6.2: Efficacy Objectives	For clarification
Text for exploratory objectives/endpoints was revised [redacted]	Synopsis: Exploratory Objectives Sections 6.2.1.3 and 6.2.2.3: Exploratory Objectives Section 14.1.1.3: Exploratory Endpoints	To algin with Appendix 4
Updated text to include that safety monitoring will continue 30 days after the EOS visit and all visits will be scheduled based on 30-day month calender	Synopsis: Methodology, Statistical Methods Section 7.1: Overall Study Design Figure 4 footnotes	To align with Section 13.3 for consistency and for clarification
Text was added to state that all developmental milestones should be assessed, documented and video recorded regardless of previous attainment	Section 11.1: Developmental Milestones	For clarification
Text added to state that additional information on contractures will also be collected, as described in the Physical Assessments Manual	Section 11.2.2: CHOP INTEND	Collection of contractures data was included in the scoring sheet provided in Appendix 6 of previous protocol versions, but is not included in the new scoring sheet provided by CHOP. The Physical Assessment Manual contains a supplemental CHOP-INTEND contractures data collection sheet for use by investigator sites, to ensure consistency in the data collected throughout the study

Description of Change	Location(s)	Rationale for Change
Text added to clarify that videos domonstrating Developmental Milestones which meet WHO and BSIDv03 criteria will be submitted to an independent, central reviewer for unbiased assessment of developmental milestone achievement	Section 11.3: Video Evidence	For clarification
Text added to include that local laboratory tests should include normal ranges appropriate for the age of the patient	Section 12.1.10: Laboratory Assessments	For clarification
Table 3 was updated to add blood sampling for CK-MB or troponin I at the EOS visit and to remove text to reduce redundancy	Table 3: Total Blood Volume: Patient	To align protocol changes and for clarification
Deleted text that is no longer applicabe within the protocol	Section 14.2: Sample Sie Calculation	For clarification
Section 14.4.1 Hy's Law Criteria and definition inserted, with a link in Section 5.5	Section 14.4: Safety Analysis	At the request of the Belgium FAMPH
Video Manual changed to Physical Assessments Manual	Throughout	The Physical Assessments Manual is the new reference document for Clinical Evaluators
Schedule of Assessments was updated with minor editting and to specify the schedule of BSIDv03 Gross and Fine Motor Subtests (with video), to include the cross-link sections, and to add CK-MB or Troponin test to the EOS visit	Appendix 1: Schedule of Assessments	To align with protocol changes and for clarification
Addition of copyright information at the bottom of each page of the Bayley record	Appendix 3: Bayley Scales of Infant and Toddler Development (Version 3)	Per copyright requirements
Scoring sheet has been replaced with an updated version	Appendix 6: CHOP INTEND	A new scoring sheet was provided by CHOP