

I concur with this review memo. I Wu 5/21/19

**FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch**

BLA NUMBER:	125694	STN #	125694.000
DATE RECEIVED BY CBER:	01-OCT-2018		
DATE REVIEW COMPLETED:	16-MAY-2019		
PRODUCT:	ZOLGENSMA (onasemnogene abeparvovec-xioi)		
APPLICANT:	AveXis, Inc.		
PROPOSED INDICATION:	Treatment of pediatric patients with infantile-onset spinal muscular atrophy (SMA) with confirmed biallelic mutations in the survival of motor neuron 1 (SMN1) gene		
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EXECUTIVE SUMMARY:

In vivo pharmacology studies of AVXS-101 were conducted in SMNdelta7 mice, a murine model of Spinal Muscular Atrophy (SMA). A single intravenous (IV) administration of AVXS-101 at dose levels ranging from 1.2×10^{13} to 1.1×10^{14} vg/kg AVXS-101 in neonatal SMNdelta7 mice resulted in dose-dependent improvement in survival. Additional studies in SMNdelta7 mice conducted using early nonclinical vector lots demonstrated improvement in motor function, neuromuscular transmission, body weight (BW) gain, and cardiac function. Improvement in survival and BW gain were highest in mice dosed at postnatal day 1 or 2.

In single-dose toxicology studies conducted in neonatal FVB mice, IV administration of AVXS-101 at dose levels of 7.9×10^{13} vg/kg and higher resulted in dose-dependent minimal to mild microscopic degeneration/regeneration of the myocardium. At dose levels 1.5×10^{14} vg/kg and higher, there were dose-dependent increases in the incidence and severity of adverse cardiac findings which included minimal to moderate atrial thrombosis, slight to marked atrial dilation, and minimal to slight fibroplasia, myocardial degeneration, and inflammation. Additional findings in the ventricles included minimal to slight inflammation, edema, and fibrosis. These findings were sometimes associated with increased heart weights and macroscopic changes which included enlarged heart, abnormal shape, and/or large atrium. Adverse findings in the liver included minimal to moderate hepatocyte degeneration/necrosis, and minimal to slight hepatocellular hypertrophy, perinuclear vacuolation, and increased Kupffer cells. Additionally, at dose levels of 2.4×10^{14} vg/kg and higher, minimal to slight perivascular and chronic inflammation were observed in the lung. AVXS-101-related mortality was observed at dose levels of 2.4×10^{14} vg/kg and higher, associated with the cardiac and liver toxicities observed. The cause of death was most frequently attributed to atrial thrombosis and was associated with atrial dilation, fibroplasia, myocardial degeneration, mononuclear cell infiltration, and hepatocellular degeneration/regeneration.

The biodistribution (BD) and SMN transgene expression profile of AVXS-101 were evaluated in neonatal FVB mice through 12 weeks. Following IV administration of 1.5×10^{14} vg/kg AVXS-101, the highest vector DNA concentration was detected in the heart, followed by the lung, liver, lumbar spinal cord, quadriceps muscle, brain, ovary, spleen, and testis. The human SMN mRNA transcripts had a similar tissue expression profile with highest levels in the heart, followed by quadriceps muscle, liver, lung, brain, and spinal cord. Low levels of SMN mRNA were detected in the spleen and gonadal tissues.

Studies to evaluate the safety pharmacology, developmental and reproductive toxicity, genotoxicity, carcinogenicity/tumorigenicity of AVXS-101 were not conducted for AVXS-101. These studies were not warranted based on the product characteristics, results from the toxicology studies, and target patient population.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies in the pharmacology-toxicology studies, and there are no outstanding requests for additional nonclinical data. The nonclinical data provided in this submission support the approval of this biologics license application.

Formulation and Chemistry:

AVXS-101 (scAAV9.CBA.SMN) is a non-replicating, self-complementary adeno-associated virus serotype 9 (scAAV9) vector at a target concentration of 2.0×10^{13} vector genomes (vg)/mL. (b) (4) of AVXS-101 solution in (b) (4) contains 20 mM Tromethamine (Tris), 1 mM magnesium chloride, 200 mM sodium chloride, and 0.005% w/v Poloxamer 188. The pH range of the solution is (b) (4). AVXS-101 is filled into 10 mL (b) (4) (b) (4) vials with a nominal fill volume of 5.5 mL or 8.3 mL and stored frozen at $\leq -60^\circ\text{C}$.

AVXS-101 is provided in a vial sealed with a sterile, ready to use, 20 mm, (b) (4), chlorobutyl elastomeric stopper. The stopper is capped with a sterile, 20 mm flip-off, aluminum seal with a colored plastic button cap.

AVXS-101 is a recombinant scAAV9 encoding for the human survival motor neuron (*SMN*) gene under the control of the cytomegalovirus (CMV) enhancer/chicken- β -actin-hybrid promoter (CBA). (b) (4)

. The vector construct is illustrated in Figure 1. (b) (4)

(b) (4)

Related File(s)

IND #15699; AveXis Inc.; Adeno-Associated Virus Serotype 9 Encoding Human Survival Motor Neuron Gene Under the Control of CMV Enhancer/Chicken Beta-Actin Hybrid Promoter, Expressing Human Survival Motor Neuron Protein (scAAV9.CV.SMN); Administered Intravenously; for Treatment of Spinal Muscular Atrophy Type 1 (SMA1) in Pediatric Patients

Abbreviations

AAV	Adeno-associated virus
ALT	Alanine aminotransferase
BD	Biodistribution
BW	Body weight
CBA	Chicken beta-actin
CK	Creatine kinase
CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
(b) (4)	
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglion/ganglia
F	Female
GFP	Green fluorescent protein
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked Immunosorbent Spot
GLP	Good Laboratory Practice
ICV	Intracerebroventricular
(b) (4)	
LV	Left ventricle/ventricular
M	Male
mRNA	Messenger ribonucleic acid
NCH	Nationwide Children's Hospital
NHP	Nonhuman primate
NOAEL	No Observed Adverse Effect Level
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PND	Postnatal day
qPCR	Quantitative polymerase chain reaction
rAAV	Recombinant adeno-associated virus
RNA	Ribonucleic acid
ROA	Route of administration
scAAV9	Self-complementary adeno-associated virus serotype 9
shRNA _{SMN}	Short hairpin ribonucleic acid targeting the survival of motor neuron gene
SMA	Spinal Muscular Atrophy
SMN	Survival of motor neuron
vg	Vector genome
vp	Viral particle
VP	Viral protein
(b) (4)	

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INTRODUCTION

AVXS-101 is a gene therapy product indicated for the treatment of pediatric patients less than 2 years of age with spinal muscular atrophy (SMA) with bi-allelic mutations in the *survival motor neuron 1 (SMN1)* gene. SMA type 1 is defined by the onset of progressive muscle weakness associated with loss of lower motor neurons prior to gaining the ability to sit independently. AVXS-101 uses a viral vector for delivery of a gene to express the functional SMN protein in cells. The recommended dose level is 1.1×10^{14} vg/kg of AVXS-101 administered by IV infusion.

NONCLINICAL STUDIES

Initial pharmacology/toxicology studies were conducted with early nonclinical vector lots manufactured using a different process from the commercial manufacturing process and vector titers were determined using a different dose determining assay. Due to inconsistencies in the previous dose determining assays, the dose levels evaluated in those studies cannot be verified or directly compared with AVXS-101 dose levels as determined by the current validated (b) (4) assay. Thus, the nonclinical studies conducted using those early vector lots are briefly summarized under “Additional Supporting Studies”. The pharmacology and toxicology studies conducted using AVXS-101 vector lots measured using the validated (b) (4) assay are reviewed in detail in the sections that follow.

Note: The following reports submitted in Module 4.2.2 ‘Pharmacokinetics’ / Module 4.2.2.1 ‘Analytical Methods and Validation Reports’ are not reviewed in detail in this memo:

- Biodistribution of AVXS-101 Utilizing FBV/NJ Neonatal Mice Intravenously Dosed using GMP Lot 816836 (Study RPT-380)
- Method for Estimating Circulating Anti-AAV9 Antibody Titer by Binding ELISA in Human Blood Samples (SOP-283)
- Method for Estimating Circulating Anti-SMN Antibody Titer by Binding ELISA in Human Blood Samples (SOP-284)
- PBMC Interferon Gamma Detection for AAV9 and SMN via ELISPOT (SOP-300)

Study RPT-380 and SOP-283 were reviewed by the Chemistry, Manufacturing and Controls (CMC) reviewers and were deemed adequate. SOP-284 and SOP-300 were assessed by this Pharmacology/Toxicology (PT) reviewer and deemed adequate.

PHARMACOLOGY STUDIES**Summary List of Pharmacology Studies**

The following pharmacology study supports the rationale for the intravenous (IV) administration of AVXS-101 in the target patient population.

Study Number	Study Title/Description	Report Number
1	In Vivo Relative Potency Assay Using the SMNdelta7 Transgenic Mouse – History of Development and Individual Mouse Survival Data	RPT-777

Overview of Pharmacology Studies**In Vivo Study in a Murine Spinal Muscular Atrophy Model****Study #1**

Report Number		RPT-777 [Source: Module 1.11.2 (BLA125694/0.3)]
Date Report Signed		October 19, 2018
Title		In Vivo Relative Potency Assay using the SMNdelta7 Transgenic Mouse – History of Development and Individual Mouse Survival Data
GLP Status		No
Testing Facility		AveXis
Objective(s)		<ol style="list-style-type: none"> To develop a robust GMP quantitative relative potency assay using the SMNdelta7 mouse model of SMA for lot disposition and stability of AVXS-101 for both clinical and commercial use. To evaluate performance comparability between AVXS-101 lots NCHAAV9SMN0613 (Phase 1) and AveXis Phase 3 clinical lots
Study Animals	Strain/Breed	SMNdelta7 (FVB genetic background) transgenic model
	Species	(b) (4)
	Age	Postnatal day (PND) 0/1
	Body Weight	1.5 grams
	#/sex/group	Not provided
	Total #	515
Test Article(s)		AVXS-101 (NCHAAV9SMN0613, 816836, 600156, 600337, 600443) Note: Lot NCHAAV9SMN0613 was the Phase 1 lot produced at Nationwide Children's Hospital. Lots 816836, 600156, 600337, and 600443 were produced by AveXis
Control Article(s)		Vehicle (b) (4)
Route of Administration		IV via the temporal vein
Description of the Disease/Injury Model and Implant Procedure		The SMNdelta7 mouse lacks the endogenous mouse <i>Smn1</i> gene but carries 2 copies of intact human <i>SMN2</i> (<i>hSMN2</i>) and an additional 2 copies of <i>hSMN2</i> with exon 7 removed, which together provide sufficient SMN expression to prevent embryonic lethality. The SMNdelta7 mouse recapitulates many features of Spinal Muscular Atrophy (SMA) such as low levels of SMN expression, motor neuron loss, weakness, and premature death.

Study Groups and Dose Levels	<p>Experiment 1 (April 2017 – December 2017)</p> <ul style="list-style-type: none"> — Group 1: Vehicle control (0 vg/kg) — Groups 2-6: NCHAAV9SMN0613 (1.2×10^{13}, 2.9×10^{13}, 7.4×10^{13}, 1.2×10^{14}, 2.9×10^{14} vg/kg) <p>Experiment 2 (June 2017 – June 2018, ongoing)</p> <ul style="list-style-type: none"> — Group 1: Vehicle control (0 vg/kg) — Groups 2-4: NCHAAV9SMN0613 (1.2×10^{13}, 2.9×10^{13}, 7.4×10^{13}, 1.2×10^{14}, 2.9×10^{14} vg/kg) — Groups 5-7: AVXS-101 Lot 816836 (1.2×10^{13}, 7.4×10^{13}, 2.9×10^{14} vg/kg) <p>Experiment 3 (July 2017 – June 2018, ongoing)</p> <ul style="list-style-type: none"> — Group 1: Vehicle control (0 vg/kg) — Groups 2-4: NCHAAV9SMN0613 (1.2×10^{13}, 7.5×10^{13}, 2.9×10^{14} vg/kg) — Groups 5-7: AVXS-101 Lot 816836 (1.2×10^{13}, 7.5×10^{13}, 2.9×10^{14} vg/kg)
Study Groups and Dose Levels	<p>Experiment 4 (September 2017 – June 2018, ongoing)</p> <ul style="list-style-type: none"> — Group 1: Vehicle control (0 vg/kg) — Groups 2-6: NCHAAV9SMN0613 (1.0×10^{12}, 1.2×10^{13}, 7.5×10^{13}, 1.1×10^{14}, 2.9×10^{14} vg/kg) — Groups 7-10: AVXS-101 Lot 600156 (1.0×10^{12}, 1.2×10^{13}, 7.5×10^{13}, 1.1×10^{14} vg/kg) — Groups 11-15: AVXS-101 Lot 816836 (1.0×10^{12}, 1.2×10^{13}, 7.5×10^{13}, 1.1×10^{14}, 2.9×10^{14} vg/kg) <p>Experiment 5 (December 2017 – June 2018, ongoing)</p> <ul style="list-style-type: none"> — Group 1: Vehicle control (0 vg/kg) — Groups 2-5: NCHAAV9SMN0613 (1.0×10^{12}, 1.2×10^{13}, 7.5×10^{13}, 1.1×10^{14} vg/kg) — Groups 6-9: AVXS-101 Lot 600307 (1.0×10^{12}, 1.2×10^{13}, 7.5×10^{13}, 1.1×10^{14} vg/kg) <p>Experiment 6 (January – June 2018, ongoing)</p> <ul style="list-style-type: none"> — Group 1: Vehicle control (0 vg/kg) — Groups 2-5: NCHAAV9SMN0613 (1.0×10^{12}, 1.2×10^{13}, 7.5×10^{13}, 1.1×10^{14} vg/kg) — Groups 6-9: AVXS-101 Lot 600443 (1.0×10^{12}, 1.2×10^{13}, 7.5×10^{13}, 1.1×10^{14} vg/kg)
Dosing Regimen	Single administration
Randomization	Yes, group selection was randomized by litter
Description of Masking	Not described
Scheduled Sacrifice Time Points	N/A

Background:

- Study #1 (RPT-777) is a summary of six independent in vivo experiments initiated in April 2017 and is still ongoing as of June 2018. The cut-off date for the survival data analyzed and presented here was May 3, 2018.

- The AVXS-101 lots evaluated and summarized in Study #1 include the clinical lots used in the AVXS-101-CL-101 Phase 1 trial (Lot NCHAAV9SMN0613) and ongoing AVXS-101-CL-303 Phase 3 trial (Lots 816836, 600156, 600307, 600443). As of June 1, 2018, there were 2 subjects dosed with AVXS-101 Lot 816836, 12 subjects with Lot 600156, 3 subjects with Lot 600307, and 3 subjects with Lot 600443. Two of the AVXS-101 lots were also evaluated in Toxicology Studies #20122446 (Lot 816836) and #8384031 (Lot 600650).
- Study #1 was conducted to bridge the relative potency of the AVXS-101 Phase 3 clinical lots to the Phase 1 lot (NCHAAV9SMN0613) produced at Nationwide Children's Hospital using the SMNdelta7 mouse model. The comparability of the Phase 1 and Phase 3 clinical lots was reviewed by the CMC reviewers.

Key Evaluations and Assessments:

- Per SOP-268, SOP-285_v3.0 and v4.0, and SOP-346_v1.0, daily monitoring of mortality or greater than 20% body weight loss were used as key endpoints. The figures and tables presented below reflect the combined survival data from each AVXS-101 lot (grouped by dose level) from the different experiments in Study #1.

Key Results:

- A single IV administration of AVXS-101 Lot NCHAAV9SMN0613 at dose levels of 1.0×10^{12} - 2.9×10^{14} vg/kg demonstrated dose-dependent improvement in survival in neonatal SMNdelta7 mice for dose levels between 1.2×10^{13} vg/kg – 1.1×10^{14} vg/kg (Figure 2 and Table 1).

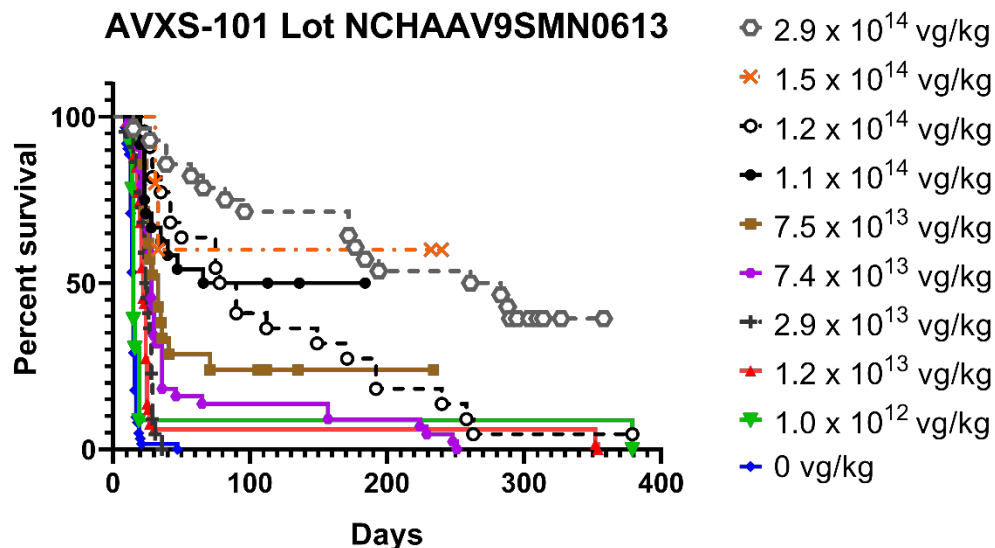


Figure 2. Kaplan-Meier plot illustrating the survival of SMNdelta7 mice following the IV administration of various dose levels of AVXS-101 (Lot NCHAAV9SMN0613).

- Single IV injection of AVXS-101 Lot 816836 in neonatal SMNdelta7 mice resulted in increased survival. Dose-dependent improvement in survival was observed at dose levels between 1.2×10^{13} vg/kg - 1.1×10^{14} vg/kg (Figure 3 and Table 1).

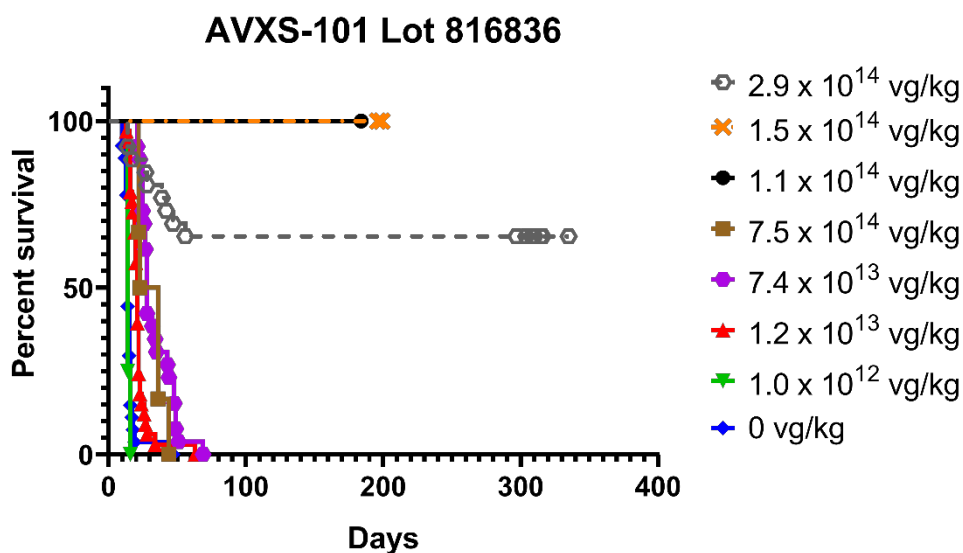


Figure 3. Kaplan-Meier plot illustrating the survival of SMNdelta7 mice following the IV administration of various dose levels of AVXS-101 (Lot 816836).

Table 1. Survival of SMNdelta7 mice administered AVXS-101 Phase 1 Lot NCHAAV9SMN0613 and Phase 3 Lot 816836

Dose level (vg/kg)	NCHAAV9SMN0613		816836	
	n	Median Survival (PND)	n	Median Survival (PND)
0	62	15	27	14
1.0×10^{12}	23	15	4	14
1.2×10^{13}	66	22	33	21
2.9×10^{13}	22	24.5	--	--
7.4×10^{13}	44	28	26	28
7.5×10^{13}	21	33	6	29.5
1.1×10^{14}	24	125	4	>184
1.2×10^{14}	22	84	--	--
1.5×10^{14}	5	>272	5	>199
2.9×10^{14}	28	272	26	>335

Reviewer Comments:

- Administration of 1.0×10^{12} vg/kg AVXS-101 did not affect survival compared to controls.

- *It is unclear whether there is further dose-dependent improvement in survival at dose levels greater than 1.1×10^{14} vg/kg.*
- *For AVXS-101 Lot 816836, only 4 and 5 animals were included in the 1.1×10^{14} vg/kg and 1.5×10^{14} vg/kg groups, respectively. Thus, the experiment did not have enough statistical power to compare the effects at the two dose levels.*
- *Limitations of these data include potential variability between litters/maternal effects, timing of vector administration, and criteria for determination of the survival endpoint (death or 20% body weight loss).*

— AVXS-101 Lot 600156 administered as a single IV injection resulted in a dose-dependent improvement in survival between dose levels 1.2×10^{13} and 1.1×10^{14} vg/kg AVXS-101 (Figure 4 and Table 2).

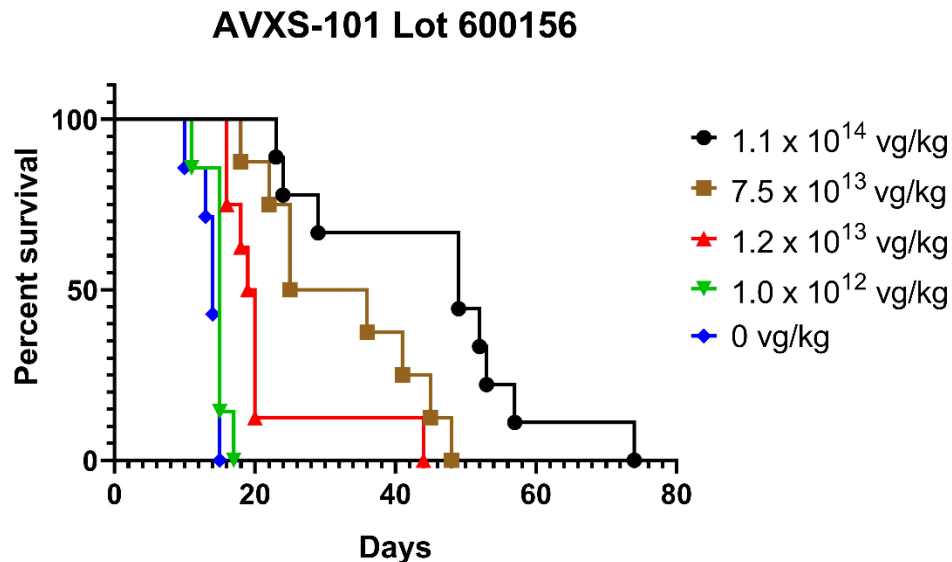


Figure 4. Kaplan-Meier plot illustrating the survival of SMNdelta7 mice following the IV administration of various dose levels of AVXS-101 (Lot 600156).

— There was a dose-dependent improvement in survival following the administration of AVXS-101 lot 600307 at dose levels 1.2×10^{13} up to 7.5×10^{13} vg/kg. Dose levels between 7.5×10^{13} and 1.1×10^{14} vg/kg trended towards an increase in median survival (Figure 5 and Table 2).

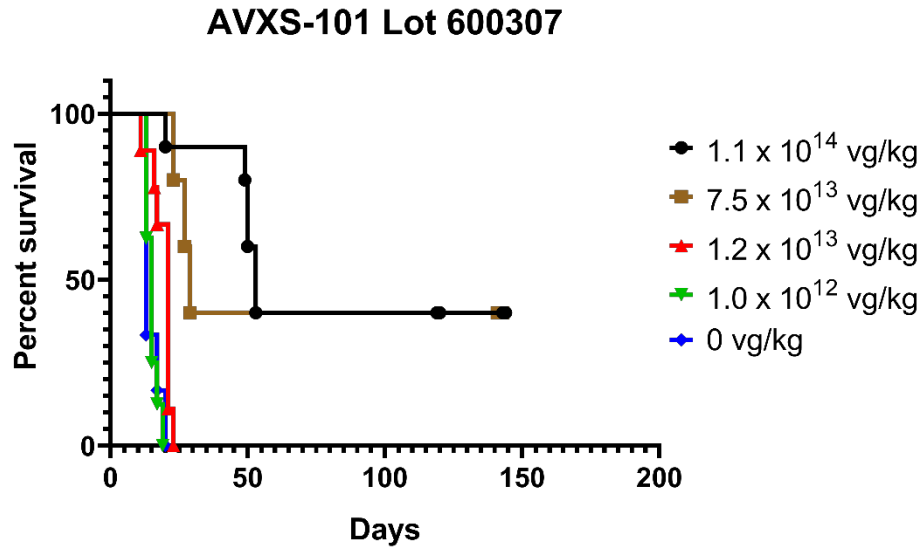


Figure 5. Kaplan-Meier plot illustrating the survival of SMNdelta7 mice following the IV administration of various dose levels of AVXS-101 (AveXis lot #600307).

— Results for AVXS-101 lot 600443 showed a trend toward dose-dependent improvement in survival at dose levels of 1.2×10^{13} up to 1.1×10^{14} vg/kg AVXS-101 (Figure 6 and Table 2).

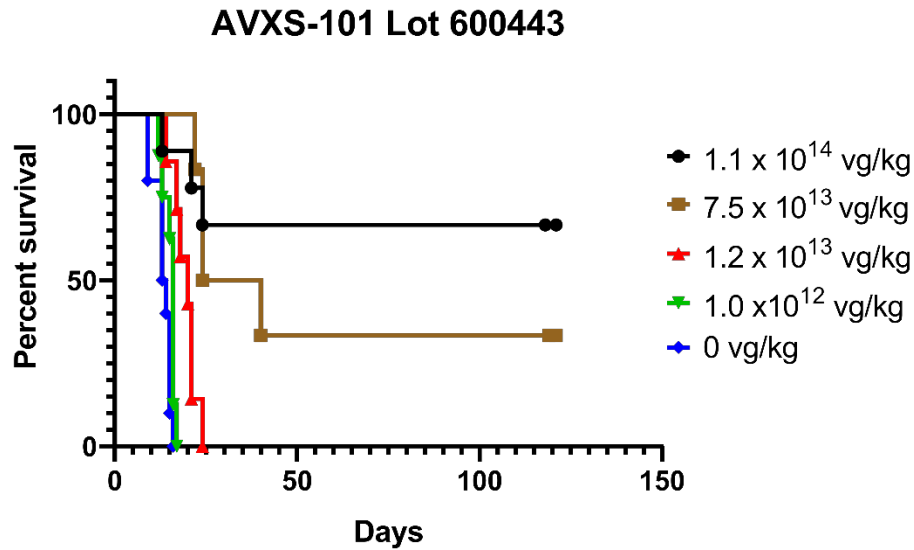


Figure 6. Kaplan-Meier plot illustrating the survival of SMNdelta7 mice following the IV administration of various dose levels of AVXS-101 (AveXis lot #600443).

Table 2. Survival of SMNdelta7 mice administered with different dose levels of AVXS-101 Lots 600156, 600307, and 600443

Dose level (vg/kg)	Lot 600156		Lot 600307		Lot 600443	
	n	Median Survival (PND)	n	Median Survival (PND)	n	Median Survival (PND)
0	7	14	6	13	10	13.5
1.0×10^{12}	7	15	8	15	8	16
1.2×10^{13}	8	19.5	9	21	7	20
7.5×10^{13}	8	30.5	5	29	6	32
1.1×10^{14}	9	49	10	53	9	>121

Reviewer Comments:

- *At the 1.1×10^{14} vg/kg dose level, the median survival of 49 days for Lot 60015 and 53 days for Lot 600307 were shorter than that observed with AVXS-101 Lots NCHAAV9SMN0613 (125 days) and 816836 (>184 days) in SMNdelta7 mice. It is unclear what the cause for the differences is. This data is limited by the small animal numbers included in each group, potential litter/maternal effects, and inter-experiment variability.*
- *The bioactivity profiles of AVXS-101 Lots 600156, 600307, and 600443 in SMNdelta7 mice were similar in the dose range between 1.0×10^{12} and 7.5×10^{13} vg/kg.*
- *At dose levels of 1.2×10^{13} up to 1.1×10^{14} vg/kg, the single IV administration of the various clinical vector lots of AVXS-101 resulted in a dose-dependent improvement in survival in neonatal SMNdelta7 mice.*
- *Additional nonclinical pharmacology data for studies conducted using early nonclinical vector lots are summarized under “Additional Supporting Studies” at the end of this review memo due to uncertainty in the actual dose levels administered. The nonclinical studies evaluated timing of vector administration, motor neuron transduction, SMN transgene expression, durability of response, and functional motor assessments in SMNdelta7 mice.*

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies were performed with AVXS-101.

PHARMACOKINETIC STUDIES (Biodistribution)

The vector biodistribution analysis in mice and NHPs were incorporated in the toxicology studies. Therefore, these data are summarized with the respective toxicology study.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

Single-dose toxicology studies were conducted to evaluate the safety of AVXS-101 following a single IV administration in healthy neonatal FVB/n mice. No repeat-dose toxicology studies were performed with AVXS-101 since this will be intended for single IV administration only.

Single-dose IV Toxicology Studies:

Study Number	Study Title	Report Number
2	AVXS-101: A Single Dose Temporal Vein Injection Study in the Neonatal FVB/NJ Mouse Followed by a 12-Week Observation Period (GLP)	20122446
3	A Single Dose of AVXS-101 (Lot Number 600443) via Temporal Vein Injection in Neonatal FVB ^{(b) (4)} Mice Followed by a 12-Week Observation Period	8384031

Developmental and Reproductive Toxicology (DART) Studies:

Per the applicant, studies were not conducted to evaluate the reproductive and developmental risk for the following reasons:

- The target patient population for AVXS-101 with infantile-onset SMA are typically less than 1 year of age and sexually immature.
- Toxicology and biodistribution studies in healthy mice showed relatively lower vector DNA levels in the gonads relative to other tissues and declined over time. Additionally, the SMN expression levels in the gonads of healthy mice and NHPs were either absent or very low at 12 weeks following vector administration.

Genotoxicity Studies:

Per the applicant, studies were not conducted to evaluate genotoxicity for the following reasons:

- Recent scientific literature supports low random integration frequencies of recombinant AAV (rAAV) vectors^{1,2} and lack of hepatic genotoxicity in rAAV2/5 genome integration in NHP and human liver samples.³

Carcinogenicity/Tumorigenicity Studies:

Per the applicant, studies were not conducted to evaluate carcinogenicity/tumorigenicity for the following reasons:

- Ongoing clinical trials (under IND #15699) since September 2013 have shown no evidence of tumor formation clinically in any subject administered with AVXS-101.

¹ Nowrouzi, A., Penaud-Budloo, M., Kaepfel, C., Appelt, U., Le Guiner, C., Moullier, P., . . . Schmidt, M. (2012). Integration frequency and intermolecular recombination of rAAV vectors in non-human primate skeletal muscle and liver. *Mol Ther*, 20(6), 1177-1186. doi:10.1038/mt.2012.47

² Chandler, R. J., Sands, M. S., & Venditti, C. P. (2017). Recombinant Adeno-Associated Viral Integration and Genotoxicity: Insights from Animal Models. *Hum Gene Ther*, 28(4), 314-322. doi:10.1089/hum.2017.009

³ Gil-Farina, I., Fronza, R., Kaepfel, C., Lopez-Franco, E., Ferreira, V., D'Avola, D., . . . Schmidt, M. (2016). Recombinant AAV Integration Is Not Associated With Hepatic Genotoxicity in Nonhuman Primates and Patients. *Mol Ther*, 24(6), 1100-1105. doi:10.1038/mt.2016.52

- Although wild-type AAV2 has been associated with clonal integration in 11 of 193 human hepatocellular carcinoma specimens occurring near known cancer driver genes,⁴ currently, there are no available scientific evidence associating AAV9 vectors with similar tumorigenic phenomenon.

Reviewer comment: *The rationales provided above are acceptable based on the product characteristics, results of the toxicology studies, and target patient population.*

Overview of Toxicology Studies

Study #2

Report Number		20122446
Date Report Signed		February 28, 2018
Title		AVXS-101: A Single Dose Temporal Vein Injection Study in the Neonatal FVB/NJ Mouse Followed by a 12-Week Observation Period
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To assess the potential toxicity and biodistribution of AVXS-101 (GMP batch, AveXis lot 816836) in the CNS when administered once via a temporal vein injection in neonatal FVB/NJ mice
Study Animals	Strain/Breed	FVB/NJ mice
	Species	(b) (4)
	Age	PND1
	Body Weight	Minimum weight of 1.0 gram at the time of dosing. Mice were on average 1.4 grams. Note: dose levels listed are based on the actual weight
	#/sex/group	45/sex/group
	Total #	360 mice (180 males and 180 females)
Test Article(s)		AVXS-101 (GMP batch, AveXis lot #816836)
Control Article(s)		(b) (4)
Route of Administration		IV via the temporal vein
Description of the Disease/Injury Model and Implant Procedure		N/A
Study Groups and Dose Levels		Group 1 – 0 vg/kg Group 2 – 7.90×10^{13} vg/kg (1.11×10^{11} vg/50 μ L/mouse) Group 3 – 2.37×10^{14} vg/kg (3.32×10^{11} vg/50 μ L/mouse) Group 4 – 3.91×10^{14} vg/kg (5.47×10^{11} vg/50 μ L/mouse)
Dosing Regimen		Single administration
Randomization		No
Description of Masking		Not described
Scheduled Sacrifice Time Points		Weeks 3, 6/7, 12

4 Nault, J. C., Datta, S., Imbeaud, S., Franconi, A., Mallet, M., Couchy, G., . . . Zucman-Rossi, J. (2015). Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas. *Nat Genet*, 47(10), 1187-1193. doi:10.1038/ng.3389

*Key Evaluations and Assessments:*In-life:

- Mortality/morbidity: Twice daily
- Clinical observations: Once daily during recovery period until weaning and weekly thereafter
- BW: Study Day 1 and twice weekly thereafter

Terminal (Weeks 3, 6/7, 12):

- Clinical pathology (hematology, clinical chemistry)
- Gross pathology, organ weights
- Histopathology: Brain, spinal cord, heart, inguinal lymph node, kidney, masseter muscle, liver, lung, ovary, pancreas, quadriceps muscle, jejunum, spleen, testes, epididymis, uterus, bronchi, diaphragm, gross lesions, and ileum
- Vector BD analysis by (b) (4) was assessed in Group 3 (2.37×10^{14} vg/kg AVXS-101) and Group 1 (control) animals. The following tissues were evaluated: brain, lumbar spinal cord, heart, liver, lung, spleen, and quadriceps muscle.

Key Results:

- Mortalities: There were 2 unscheduled deaths recorded in Group 1 on Day 8 and Day 80, one presumed cannibalized and another with undetermined cause of death. No mortalities were observed in Groups 2 and 3. There were 30 unscheduled deaths related to AVXS-101 administration in Group 4 between Days 7 and 47. Thirteen deaths were attributed to heart thrombi, 16 deaths had undetermined causes, and 1 from a presumed cannibalization by the dam. There were no gross lesions observed in any of these mice. However, all 30 deaths had associated minimal or mild microscopic degeneration/regeneration in the heart. In addition, 20 of these mice had microscopic hepatocellular regeneration and mild to moderate degeneration. Cardiac and liver histopathologic findings were considered related to AVXS-101 administration.
- Clinical observations: There were no test article-related abnormal clinical observations in Groups 2 and 3. Adverse clinical observations were recorded in Group 4 between Weeks 3 and 7, which included decreased activity, abdominal distension, dehydration, hunched posture, ungroomed fur, labored or shallow breathing, convulsions, cold to touch, thin body, dark eyeball, and shut eyes.
- BW: Group 4 mice had reduced BW gain at Days 21-25 and Days 25-29. There were no test article-related adverse changes in mean BWs in any other groups.
- Hematology: Minimal to moderate changes in white blood cells (1.6 to 2.2-fold increase), monocytes (1.5 to 2.0-fold increase), eosinophils (2.0 to 4.0-fold increase), reticulocytes (0.1 to 0.2-fold decrease) and lymphocytes (1.7 to 2.4-fold increase) were reported in Groups 2-4 at Week 3. These changes were not dose-dependent.
- Clinical chemistry: Minimal to moderate increases (1.2 to 2.2-fold change) in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in Group 4 males and

females (Weeks 3 and 7). Minimal increases in ALT (1.3 to 1.4-fold change) were also observed in Group 2 males (Week 12), and Group 3 males (Week 12) and females (Week 7). Minimal increases in AST (1.5-fold change) was also observed in Group 3 females (Week 7). Elevated creatinine kinase (CK) (3.6-fold change) was observed in Group 4 females (Week 7). Minimal to marked increases in urea (1.2 to 1.6-fold change), creatinine (1.5 to 6-fold change), potassium (1.1 to 1.4-fold change), and phosphate (1.1 to 1.2-fold change) were observed in Groups 3 and 4 at Weeks 3 and 7. Per the study report, these changes were suggestive of a decreased glomerular filtration rate but had no microscopic correlates.

- Gross pathology, organ weights: There were no test article-related findings.
- Histopathology: Test article-related findings in the heart included dose-dependent minimal to mild degeneration/regeneration in Groups 2-4 starting at Week 3, with decrease in the severity of the lesions to minimal at Week 12 for Groups 2 and 3 (no animals in Group 4 were available for analysis at Week 12 due to mortality). Microscopic degeneration/regeneration was observed most prominently in the myocardium along the endocardial surface of the ventricular free walls, interventricular septum, bases of papillary muscles, and atrial walls. Minimal to moderate atrial thrombi were observed in in Group 4 animals that died prematurely and at scheduled sacrifice at Weeks 6/7.

Minimal to moderate liver degeneration and minimal regeneration was observed in Group 4 animals at Weeks 3 and 6/7 and for unscheduled mortalities. Minimal regeneration characterized by increased binuclear hepatocytes, mitotic figures, karyomegaly, and hepatocyte hypertrophy was also observed in Group 3 females at Week 3. Minimal degeneration was also observed in several Group 2 and Group 3 animals at Weeks 3 and 6/7. Per the study pathologist, they were of a severity and appearance commonly seen in control group animals.

- BD analysis: Vector DNA was detected through Week 12 in all tissues evaluated for Group 3. The highest vector concentration was detected in the heart, followed by the lung, liver, lumbar spinal cord, quadriceps muscle, brain, and spleen (Table 3).

Table 3. AVXS-101 DNA biodistribution in FVB/NJ mice (Group 3)

Organ	Week 3 vg/ μ g DNA	Week 7 vg/ μ g DNA	Week 12 vg/ μ g DNA
Brain	25,119	6,913	7,048
Heart	ND	188,644	169,098
Liver	ND	38,705	24,670
Lumbar spinal cord	32,823	19,789	13,651
Lung	ND	99,116	35,588
Quadriceps muscle	ND	11,694	13,017
Spleen	ND	129	181

ND: No data provided

- Human SMN transgene expression was detected in the brain, lumbar spinal cord, heart, liver, lung, spleen, and quadriceps in Group 3 mice at 12 weeks following AVXS-101 administration. SMN mRNA transcript levels were the highest in the heart and quadriceps muscle and lowest in the spleen (Table 4).

Table 4. Human SMN mRNA expression in FVB/NJ mice (Group 3)

Organ	Week 3 copies/ μ g total RNA	Week 7 copies/ μ g total RNA	Week 12 copies/ μ g total RNA
Brain	64,350	49,975	59,700
Heart	ND	1,724,475	3,140,000
Liver	ND	12,700	35,650
Lumbar spinal cord	59,100	12,330	28,738
Lung	ND	53,775	18,085
Quadriceps muscle	ND	1,322,250	1,149,250
Spleen	ND	191	254

ND: No data provided

Reviewer Comments:

- Due to the unscheduled mortalities in Group 4, there were no mice available for in-life and post-mortem assessments after the Week 6/7 time point.*
- Mortality and adverse clinical signs were observed in mice administered the highest dose level of 3.91×10^{14} vg/kg AVXS-101 (Group 4). The cause of death for 13 mice was attributed to formation of thrombi in the heart, while the remaining 16 deaths were undetermined. The pathogenesis of atrial thrombus formation in Group 4 animals was not known. Based on these cardiotoxicity findings in this study for AVXS-101 Lot 816836, additional cardiac monitoring was incorporated in the clinical protocols, including electrocardiogram, echocardiograms, and serum cardiac biomarkers.*
- Increased ALT, AST and/or CK through 12 weeks were associated with muscular and/or hepatocellular damage as observed microscopically as degeneration/regeneration in the heart and degeneration/regeneration in the liver.*
- Given the changes in hematology and clinical chemistry parameters and the presence of minimal myocardial degeneration/regeneration in the heart at Week 12 in mice administered the lowest dose level of 7.9×10^{13} vg/kg AVXS-101, a NOAEL was not identified in this study. The low dose level used in this study is lower than the recommended clinical dose level of 1.1×10^{14} vg/kg.*

Study #3

Report Number		8384031
Date Report Signed		September 25, 2018
Title		A Single Dose of AVXS-101 (Lot Number 600443) via Temporal Vein Injection in Neonatal FVB/ ^{(b) (4)} Mice Followed by a 12-Week Observation Period
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To assess the toxicity and BD profile of AVXS-101 (Lot Number 600443) following a single temporal vein injection in neonatal FVB/ ^{(b) (4)} mice.
Study Animals	Strain/Breed	FVB/ ^{(b) (4)}
	Species	(b) (4)
	Age	PND0
	Body Weight	1.1 – 1.7 grams (Average of 1.5 grams)
	#/sex/group	47-49/sex/group
	Total #	384
Test Article(s)		AVXS-101 (GMP batch, AveXis lot #600443)
Control Article(s)		(b) (4) buffer (20mM Tris, 1 mM MgCl ₂ , 200 mM NaCl, 0.005% Poloxamer 188, pH 8.0 (b) (4) (Lot #600650)
Route of Administration		IV via the temporal vein
Description of the Disease/Injury Model and Implant Procedure		N/A
Study Groups and Dose Levels		Group 1 – 0 vg/kg (n=96) Group 2 – 1.5×10^{14} vg/kg (2.2×10^{11} vg/50 μ L/mouse) (n=96) Group 3 – 2.4×10^{14} vg/kg (3.6×10^{11} vg/50 μ L/mouse) (n=96) Group 4 – 3.0×10^{14} vg/kg (4.5×10^{11} vg/50 μ L/mouse) (n=96)
Dosing Regimen		Single administration
Randomization		Yes
Description of Masking		Not described
Scheduled Sacrifice Time Points		Week 3, 6, 12

*Key Evaluations and Assessments:*In-life:

- Mortality/morbidity: Twice daily
- Clinical observations: Twice daily cageside observations, weekly detailed observations
- BW: Day 0, weekly thereafter
- Food consumption: Day 21, weekly thereafter

Terminal (Weeks 3, 6, and 12):

- Clinical pathology (hematology, clinical chemistry, coagulation)
- Gross pathology, organ weights
- Histopathology: Brain, diaphragm, gross lesions or masses, heart, kidney, liver, lung with bronchi, inguinal lymph node, masseter muscle, quadriceps muscle, ovary, small intestine, spinal cord, spleen, testes, epididymis, and uterus

- BD analysis by (b) (4): same tissues selected for histopathology with the exception of the diaphragm, gross lesions or masses, and small intestine

Key Results:

- Mortalities: There were unscheduled deaths in Groups 1 (4), 2 (3), 3 (6), and 4 (14). Dose-dependent AVXS-related mortality was reported in 13 animals in Groups 3 and 4, associated with atrial thrombosis and atrial wall changes including dilation, fibroplasia, myocardial degeneration/necrosis and/or mononuclear cell inflammation. Liver findings of hepatocellular hypertrophy and/or decreased sinusoidal macrophages was observed in Groups 3 and 4 and individual hepatocyte necrosis in Group 4. Perivascular inflammation in the lung was also observed in Group 4 animals. A specific cause of the moribund condition or death was not determined in the other animals. A total of 5 animals were found dead or cannibalized in Groups 2 (2), 3 (2), and 4 (1) between Days 0 and 21 and were considered unrelated to AVXS-101 by the study pathologist as they occurred with no other clinical or macroscopic findings.
- Clinical observations: Adverse AVXS-101-related clinical observations in Group 4 animals (14 of 47 males and 6 of 49 females) included rough haircoat, labored respiration, hypoactivity, thin and hunched appearance, eye protrusion, and discolored eyes between Days 28-84.
- BW and food consumption: AVXS-101-related dose-dependent decrease in mean BW and BW gain for both male and female mice (reduction of $\leq 7.7\%$ compared to controls) at various timepoints during the study. A dose-dependent reduction in food consumption was also observed (-16 to -8.3%) in all male mice administered with AVXS-101 from Days 21-84 and in Groups 3 and 4 female mice between Days 35-42 (-6.5%) and Days 28-63 (-11%), respectively. Per the study report, based on the magnitude of changes in BW, BW gain, and food consumption, they were considered test article-related but not adverse.
- Hematology, coagulation, and clinical chemistry: There was a test-article related increase in neutrophil count (1.5 to 2.1-fold difference) in Group 3 and 4 males. Mild to marked decrease in platelet count was observed in a few animals in Groups 2, 3, and 4 but was considered likely related to blood collection due to the absence of a dose response according to the study pathologist. Mild to marked elevation in ALT, AST, and CK were noted at various time points in all groups. Per the study report, these observations were considered related to muscle and/or liver damage associated with study procedures and were considered not adverse as similar changes were sometimes noted in controls and findings were not dose-dependent. There were no other AVXS-101-related hematology, coagulation, and clinical chemistry findings.
- Gross pathology: Group 4 mice were found to have enlarged hearts, abnormal shape, and atrial dilation at Weeks 6 and 12. These gross findings were associated with microscopic observations of fibrosis of the ventricular myocardium, atrial thrombus, dilation, fibroplasia, myocardial degeneration/necrosis, and mononuclear immune infiltration. One

animal Group 2 (1 unscheduled death) and 2 animals in Group 4 (1 unscheduled death and one terminal sacrifice at Week 6) had small thymus, which correlated with microscopic findings of decreased lymphocytes.

- Organ weights: Increased adrenal gland and heart weights were observed in Group 4 male mice at Weeks 6 and 12, respectively.
- Histopathology: AVXS-101-related findings included dose-dependent ventricular myocardial edema, mononuclear cell inflammation, fibrosis and atrial mononuclear cell inflammation in Groups 2, 3, and 4 at Weeks 3, 6, and 12. Atrial thrombosis, dilation, fibroplasia, and myocardial degeneration/necrosis were observed in Groups 3 and 4 at Weeks 6 and 12. Cardiac findings were primarily minimal to slight in nature, with a few reported findings of moderate to marked atrial thrombus and dilation. Microscopic findings in the liver at Weeks 6 and 12 included minimal hepatocellular hypertrophy in Groups 2, 3, and 4 and increased Kupffer cells, individual necrosis, and perinuclear vacuolation of hepatocytes that were minimal to slight in severity in Groups 3 and 4. In the lung, minimal to slight perivascular inflammation and/or slight chronic inflammation was observed in Groups 3 and 4 at Week 6. Decreased lymphocytes in the thymus and/or lymphocyte necrosis were observed in one animal found in moribund condition in Group 3 and in Group 4 animals and were considered stress-related by the study pathologist.
- BD analysis: Vector DNA was detected through Week 12 in all tissues evaluated for Group 2 with the highest vector concentration in the heart and followed by the lung, liver, quadriceps muscle, lumbar spinal cord, brain, ovary, spleen, and testis (Table 5).

Table 5. AVXS-101 DNA biodistribution in FVB^{(b) (4)} mice (Group 2)

Organs	Week 3 vg/μg DNA	Week 6 vg/μg DNA	Week 12 vg/μg DNA
Brain	4,277	16,345	5,785
Heart	176,750	169,500	355,750
Liver	64,050	19,940	22,975
Lumbar spinal cord	17,538	11,465	11,370
Lung	36,175	41,500	49,700
Quadriceps muscle	13,552	7,148	18,550
Spleen	180	71	89
Ovary (n=2)	268	215	532
Testis (n=2)	130	45	27

- Human SMN transgene expression was detected in the brain, lumbar spinal cord, heart, liver, lung, spleen, and quadriceps in Group 2 mice at least 12 weeks following administration of AVXS-101. SMN mRNA transcript levels were highest at Week 3 in the heart and in quadriceps muscle, lowest in the spleen and ovary, and declined over time (Table 6).

Table 6. Human SMN mRNA expression in FVB/^(b) (4) mice (Group 2)

Organs	Week 3 copies/ μ g total RNA	Week 6 copies/ μ g total RNA	Week 12 copies/ μ g total RNA
Brain	63,550	35,128	4,121
Heart	4,547,500	2,385,000	121,975
Liver	194,250	66,150	4,945
Lumbar spinal cord	30,600	32,175	2,070
Lung	89,900	6,818	506
Quadriceps muscle	508,600	623,500	30,260
Spleen	136	72	74
Ovary (n=2)	179	123	-
Testis (n=2)	1,130	365	16

Reviewer Comments:

- *Adverse test article-related findings in mice administered 1.5×10^{14} , 2.4×10^{14} and 3.0×10^{14} vg/kg AVXS-101 included histopathology findings in the heart and liver. These were associated with macroscopic changes in the heart in animals in the 3.0×10^{14} vg/kg group. Additional microscopic findings in the lung, spleen, and thymus were observed in the two highest dose levels administered. The NOAEL was not identified for this study.*
- *Mice that were found dead had incomplete histopathologic assessments due to tissue autolysis or degradation and therefore, the relation to test article effects have not been established. Additionally, mice that were sacrificed in moribund condition or found dead prior to Day 21 were not examined post-mortem according to the protocol. This included four AVXS-101 administered animals and the relation to test article was not determined.*
- *Microscopic and gross cardiac findings related to AVXS-101 administration were observed at Weeks 6, 12 and during unscheduled sacrifice. Unscheduled mortalities were most often attributed to atrial thrombi when a cause of death could be determined. The severity of findings was generally increased in male mice compared to females.*
- *The vector DNA and transgene expression profiles of AVXS-101 lot 600443 (Study #8384031) and lot 816836 (Study #201224462) were similar with the highest vector concentrations detected in the heart, followed by the liver, lung, lumbar spinal cord, quadriceps muscle, brain and spinal cord, and low levels observed in the spleen and gonadal tissues for at least 12 weeks post-dose.*
- *AVXS-101 vector DNA had different BD kinetic profiles in the different organs evaluated. SMN transgene expression profile in all tissues generally decreased over time for this study.*

ADDITIONAL SUPPORTING STUDIES**Summary List of Additional Pharmacology Studies**

The following pharmacology studies were conducted to support the scientific rationale for the administration of AVXS-101.

Intravenous Pharmacology Studies in Healthy Animals

Study Number	Study Description (Publication Title)	Publication Citation
4	Single IV Dose (GFP) in Healthy PND1/2 C57Bl/6 Mice (Intravascular AAV9 Preferentially Targets Neonatal Neurons and Adult Astrocytes)	Foust et al, Nat Nat Biotechnol 2009; 27(1), 59-65
5	Single IV Dose (GFP) in 3-Year Old Cynomolgus Monkeys (Systemic Gene Delivery in Large Species for Targeting Spinal Cord, Brain, and Peripheral Tissues for Pediatric Disorders)	Bevan et al, Mol Ther 2011; 19(11), 1971-1980
6	Single IV Dose (GFP) in Neonatal Cynomolgus Monkeys (Rescue of The Spinal Muscular Atrophy Phenotype in A Mouse Model by Early Postnatal Delivery of SMN)	Foust et al, Nat Biotechnol 2010; 8(3), 271-274

Intravenous Pharmacology Studies in an Animal Model of Spinal Muscular Atrophy

Study Number	Study Description (Publication Title)	Report Number or Publication Citation
7	Single IV Dose (AVXS-101) in SMNdelta7 Mice (Rescue of the Spinal Muscular Atrophy Phenotype in a Mouse Model by Early Postnatal Delivery of SMN)	Foust et al, Nat Biotechnol 2010; 8(3), 271-274
8	Single IV Dose (AVXS-101) in SMNdelta7 Mice to Evaluate Cardiac Function (Early Heart Failure in the SMNdelta7 Model of Spinal Muscular Atrophy and Correction by Postnatal scAAV9-SMN Delivery)	Bevan et al, Hum Mol Genet 2010; 19(20), 3895-3905

Intrathecal Pharmacology Studies

Study Number	Study Description (Publication Title)	Report Number or Publication Citation
9	ICV or IT Dose (GFP) in 5-Day Old Piglets (Systemic Gene Delivery in Large Species for Targeting Spinal Cord, Brain, and Peripheral Tissues for Pediatric Disorders)	Bevan et al, Mol Ther 2011; 19(11), 1971-1980

Study Number	Study Description (Publication Title)	Report Number or Publication Citation
10	IT Dose (GFP) in 1-Yr Old Cynomolgus Monkeys (Improving Single Injection CSF Delivery of AAV9-Mediated Gene Therapy for SMA: A Dose-Response Study in Mice and Nonhuman Primates)	Meyer et al, Mol Ther 2015; 23(3), 477-487
11	Single ICV Dose (AVXS-101) in SMNdelta7 Mice (Improving Single Injection CSF Delivery of AAV9-Mediated Gene Therapy for SMA: A Dose-Response Study in Mice and Nonhuman Primates)	Meyer et al, Mol Ther 2015; 23(3), 477-487
12	Single ICV Dose (AVXS-101) In SMN KO (scAAV9-shRNA _{SMN}) Model in 5-Day Old Piglets (A Large Animal Model of Spinal Muscular Atrophy and Correction of Phenotype)	Duque et al, Ann Neurol 2015; 77(3), 399-414

Note: Study Nos. 4-8 are briefly summarized in the following section of this review memo under “Overview of Additional Pharmacology Studies.” Study Nos. 9-12 are not summarized in this review memo because they contained data that are not directly applicable to the intended clinical use of AVXS-101 due to a different route of administration. The vector lots used in the studies listed here differed in the manufacturing processes and vector concentration assays from the clinical vector lots subject to this BLA. Therefore, the dose levels cannot be directly compared to AVXS-101 dose levels evaluated in the pharmacology/toxicology studies reviewed above and in the clinical trials. The early versions of the AVXS-101 vector used in these studies are denoted as scAAV9.CBA.SMN.

Overview of Additional Pharmacology Studies

Intravenous Pharmacology Studies in Healthy Animals

Study #4: Single IV dose (GFP) in healthy PND1/2 C57Bl/6 mice – Foust, K. D., Nurre, E., Montgomery, C. L., Hernandez, A., Chan, C. M., & Kaspar, B. K. (2009). Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol, 27(1), 59-65. doi:10.1038/nbt.1515

Synopsis:

- This study compared the tissue BD and transduction efficiency of a recombinant scAAV9 encoding for GFP (scAAV9.CBA.GFP) following IV administration in neonatal and adult C57Bl/6 mice at dose levels of 2.7×10^{14} vg/kg and higher. GFP expression was detected in the heart, skeletal muscle, spinal cord, and brain of both neonatal and adult mice.
- The overall distribution pattern of scAAV9.CBA.GFP transduction in the CNS was characterized as mainly neuronal in neonatal mice, targeting the DRG and lower motor neurons, whereas it was mostly astrocytic in adult mice, with targeting in the brain and spinal cord.

Reviewer Comment:

The tissue/cell transduction profile of IV-administered scAAV9 appears to change based on the age of mice, with preferential transduction of motor neurons in neonates and glial cells in adult mice. This study suggests that IV dosing would need to occur during the neonatal stage in mice in order to target neuronal cells in the CNS.

Study #5: Single IV dose (GFP) in 3-year old cynomolgus monkeys – Bevan, A. K., Duque, S., Foust, K. D., Morales, P. R., Braun, L., Schmelzer, L., ... Kaspar, B. K. (2011). Systemic gene delivery in large species for targeting spinal cord, brain, and peripheral tissues for pediatric disorders. Mol Ther, 19(11), 1971-1980. doi:10.1038/mt.2011.157

Synopsis:

- This study evaluated the transgene expression profile in cynomolgus macaques of various ages (from newborn to 3 years old) following systemic administration of $1.0 - 3.0 \times 10^{14}$ vg/kg scAAV9.CBA.GFP.
- IV infusion of scAAV9.CBA.GFP in neonatal to 3-month-old cynomolgus macaques resulted in GFP expression in the CNS, skeletal muscles, liver, heart, adrenal gland, spleen, smooth muscle of the gut, testis, lungs, and kidneys at 21 to 25 days post-vector administration. Specifically, GFP expression in the CNS was detected in the motor neurons in all spinal cord segments and in the DRG.
- Cynomolgus monkeys of various ages from PND1 to 3 years old at the time of scAAV9.CBA.GFP administration exhibited primarily microglial and astrocytic cell transduction but scarce neuronal transduction in the brain.

Reviewer Comment:

The IV administration of scAAV9.CBA.GFP in neonatal to 3-month old NHPs resulted in transduction of motor neurons in the spinal cord, similar to that observed in neonatal mice, thus supporting the rationale for systemic delivery of scAAV9 to target motor neurons. However, in the brains of NHPs (regardless of age) there was a lack of scAAV9 transduction of motor neurons following systemic vector delivery. This finding of primarily glial cell transduction and minimal neuronal transduction in NHP brain was similar to the transduction pattern of IV scAAV9 in adult mice and different from the neuronal cell transduction observed in the neonatal mouse brain (Foust et al, 2009).

Study #6: Single IV dose (GFP) in neonatal cynomolgus monkeys – Foust, K. D., Wang, X., McGovern, V. L., Braun, L., Bevan, A. K., Haidet, A. M., ... Kaspar, B. K. (2010). Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat Biotechnol, 28(3), 271-274. doi:10.1038/nbt.1610

Synopsis:

- This study evaluated the IV bolus administration of scAAV9.CBA.GFP at a dose level of 1.0×10^{14} viral particles total (2.2×10^{11} vp/gram BW) in PND1 cynomolgus macaques. After 25 days, GFP expression was detected in the DRG and motor neurons in the spinal cord.

Reviewer Comment:

The results of this study provide additional support for the IV route of administration for targeting spinal motor neurons.

Note: Study #6 was reported in the same publication as Study #7 which described the systemic administration of scAAV9.CBA.SMN in a mouse model of SMA. Studies #6 and #7 are reviewed separately in this memo to distinguish the two studies, one involving scAAV9.CBA.GFP in NHP and the other using scAAV9.CBA.SMN in a disease model of SMA.

Intravenous Pharmacology Studies in an Animal Model of Spinal Muscular Atrophy

Study #7: Foust, K. D., Wang, X., McGovern, V. L., Braun, L., Bevan, A. K., Haidet, A. M., . . . Kaspar, B. K. (2010). Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat Biotechnol, 28(3), 271-274. doi:10.1038/nbt.1610

Synopsis:

- This study evaluated the IV administration of 3.3×10^{14} vg/kg scAAV9.CBA.SMN in PND1 SMNdelta7 mice. Mice were assessed for transduction in the CNS and muscle, general motor function, overall survival, BW gain, neuromuscular transmission in the tibialis anterior muscle, and determination of the optimal timing of vector administration.
- At PND10 (9 days post-administration), 42% of motor neurons in the lumbar spinal cord were transduced which correlated with increased SMN protein levels in the brain, spinal cord, and quadriceps muscle. Righting reflex evaluated at PND13 showed significant improvement in 9 of 10 mice that was >20 seconds faster than control groups, but still lagging by 10 seconds compared to wild-type littermates. Neuromuscular transmission and locomotor tests showed comparable performance to the wild-type controls. scAAV9.CBA.SMN administered mice also achieved steady gains in BW that stabilized at half the weight of wild-type controls and improved survival was also observed compared to SMA mice that received scAAV9.CBA.GFP (Figure 7).
- The administration of scAAV9.CBA.SMN at PND2 resulted in similar survival and BW gains observed when administered at PND1. However, survival and BW gains declined or were lost when AVXS-101 was administered at PND5 or later (Figure 8).

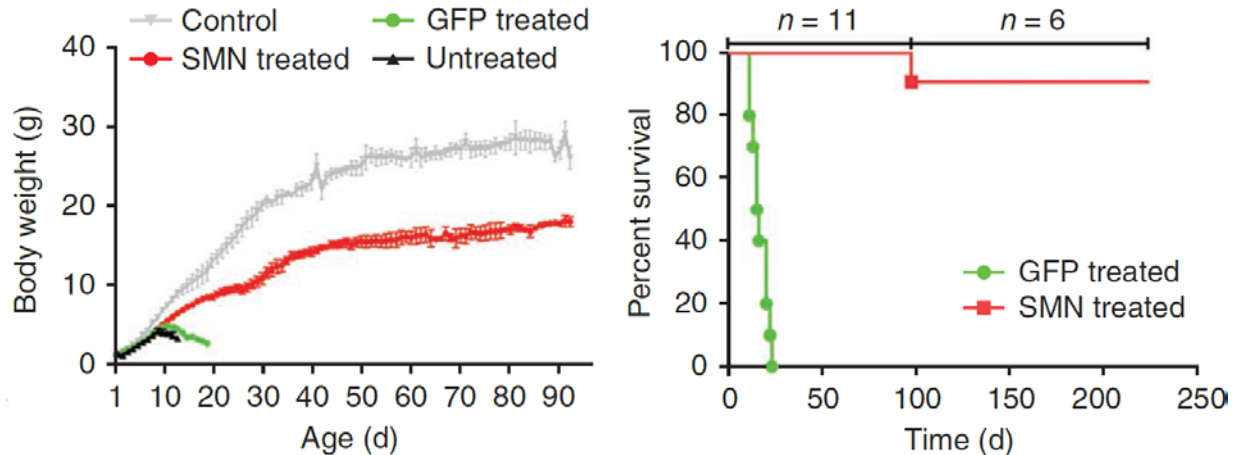


Figure 7. Body weight measurements (left panel) and survival (right panel) for SMNdelta7 mice administered scAAV9.CBA.SMN and scAAV9.CBA.GFP.

Source: Nat Biotechnol, 28(3), 271-274. doi:10.1038/nbt.1610

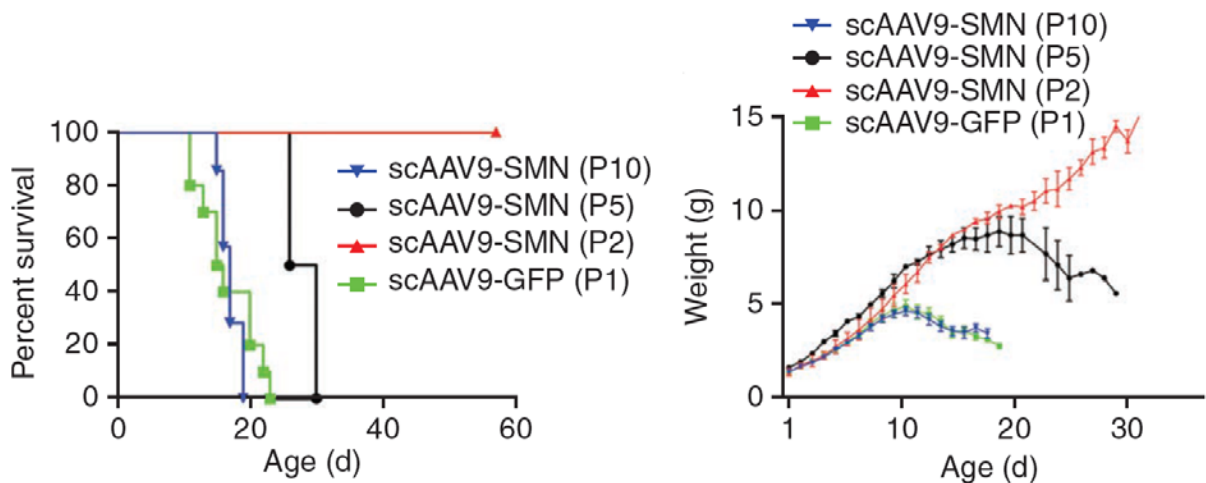


Figure 8. Kaplan-Meier plot (left panel) and body weight measurements (right panel) of SMNdelta7 mice administered with systemic scAAV9.CBA.SMN between postnatal day 1 through 10. **Source:** Nat Biotechnol, 28(3), 271-274. doi:10.1038/nbt.1610

Reviewer Comment:

This study provides scientific support for the early administration of scAAV9.CBA.SMN for achieving longer survival and greater improvement of motor functions.

Study #8: Single IV dose (AVXS-101) in SMNdelta7 mice to evaluate cardiac function – Bevan, A. K., Hutchinson, K. R., Foust, K. D., Braun, L., McGovern, V. L., Schmelzer, L., . . . Kaspar, B. K. (2010). Early heart failure in the SMNdelta7 model of spinal muscular atrophy and correction by postnatal scAAV9-SMN delivery. Hum Mol Genet, 19(20), 3895-3905. doi:10.1093/hmg/ddq300

Synopsis:

- SMNdelta7 mice have characteristic abnormal heart dimensions and cardiac dysfunction. This study evaluated the cardiac structure and function of SMNdelta7 mice following the IV administration of 3.3×10^{14} vg/kg scAAV9.CBA.SMN. At PND7 and PND14, mice administered with IV scAAV9.CBA.SMN had improved left ventricular (LV) mass, wall thickness, LV wall thickness-LV wall diameter in diastole ratio, heart rate, cardiac output, LV systolic function, and myocardial performance index.

Reviewer Comment:

This study provides support for the potential of IV administered scAAV9.CBA.SMN to improve cardiac function which may be beneficial as cardiac involvement has been reported for SMA.

Summary List of Additional Toxicology Studies

The following additional toxicology studies were conducted to evaluate the safety of scAAV9.CBA.SMN.

Single-dose IV Toxicology Studies

Study Number	Study Title / Publication Citation	Report Number
13	24-Week Toxicity and Biodistribution Study of the Self-Complementary Serotype 9 Adeno-Associated Viral Vector scAAV9.CBA.SMN in FVB Neonatal Mice	AD49TV.7A32.BTL
14	Analysis of Vector Safety Following a Single Systemic Delivery of scAAV9.CBA.SMN into Wild-type Mice	NCH Safety analysis of scAAV9.CBA.SMN in FVB mice 4.2.3.1
15	Analysis of Vector Safety Following a Single Systemic Delivery of scAAV9.CBA.SMN into (b) (4) Macaques	NCH Safety analysis of scAAV9.CBA.SMN in NHP 4.2.3.1

Single-dose ICV/Intrathecal Toxicology Studies

Study Number	Study Title / Publication Citation	Report Number
16	3-, 6-, 12-week Safety Study of CSF Delivered Self-complementary Serotype 9 Adeno-Associated Viral Vector scAAV9.CBA.SMN in FVB Neonatal Mice	CSF-AAV9-SMN-MOUSE 002
17	24-Week Safety Study of CSF Delivered Self-complementary Serotype 9 Adeno-Associated Viral Vector SCAAV9.CBA.SMN In FVB Neonatal Mice	CSF-AAV9-SMN-MOUSE 001
18	Safety Study of CSF Delivered Self-Complementary Serotype 9 Adeno-Associated Viral Vector scAAV9.CBA.SMN in (b) (4) Macaques	CSF-AAV9-SMN-NHP-001

Note: Study Nos. 13-15 are briefly summarized in this review memo under “Overview of Additional Toxicology Studies.” Study Nos. 16-18 are not summarized in this review memo

because they contained data that are not directly applicable to the intended clinical use of AVXS-101 due to the use of a different route of administration.

Overview of Additional Toxicology Studies

Single-dose IV Toxicology Studies

Study #13: 24-Week Toxicity and Biodistribution Study of the Self-Complementary Serotype 9 Adeno-Associated Viral Vector scAAV9.CBA.SMN in FVB Neonatal Mice (AD49TV.7A32.BTL)

Synopsis:

- This 24-week GLP toxicology study evaluated the safety and BD of single IV administration of scAAV9.CBA.SMN (Lot No. AAV9SMN0212-P1) at dose levels of 6.7×10^{13} and 3.3×10^{14} vg/kg in neonatal FVB mice. There were no test article-related mortalities or findings from clinical observations. However, there was a dose-dependent transient decrease in BW in male animals at both dose levels and in females administered the high dose. Male mice were more sensitive to scAAV9.CBA.SMN-related BW decrease than females. There were no scAAV9.CBA.SMN-related changes in clinical and anatomic pathology parameters. scAAV9.CBA.SMN-administered mice did not develop an antibody response against the human SMN transgene while anti-AAV9 capsid antibodies were detected at 12 and 24 weeks post-administration. The NOEL for this study was 3.3×10^{14} vg/kg.
- In mice administered 3.3×10^{14} vg/kg scAAV9.CBA.SMN, the heart had the highest concentration of vector DNA followed by the brain, liver, lung, lymph node, injection site (masseter muscle), quadricep muscle and spinal cord and the levels generally declined between the week 3 through week 24 time points assessed. Intermediate levels of vector DNA were detected in jejunum, kidney, pancreas, and spleen, while the lowest vector DNA concentration was found in the gonads.
- Human SMN transgene was highly expressed in the brain, heart, liver, spinal cord, lung, quadriceps, and the kidney of both male and female mice that were administered 3.3×10^{14} vg/kg scAAV9.CBA.SMN. The gonads were the only tissue assessed without any SMN transcriptional activity at all time points.

Reviewer Comment:

The toxicology profile of scAAV9.CBA.SMN from Study #13 differed from the results observed in Study #2 (20122446) and Study #3 (8384031). The scAAV9.CBA.SMN vector lot (AAV9SMN021212-P1) used for this study was manufactured using a different process from the AVXS-101 clinical vector lots subject to this BLA. Additionally, the uncertainty regarding the vector concentration of AAV9SMN021212-P1 limits the interpretation of this data and no conclusions on the safety of these vector dose levels or comparisons to subsequent AVXS-101 vector lots can be made from this study.

Study #14: Analysis of Vector Safety Following a Single Systemic Delivery of scAAV9.CBA.SMN into Wild-type Mice (NCH Safety analysis of scAAV9.CBA.SMN in FVB mice, Module 4.2.3.1)

Synopsis:

- This study evaluated the toxicology profile of a single IV administration of scAAV9.CBA.SMN at a dose level of 3.3×10^{14} vg/kg in healthy neonatal FVB mice through 180 days. There were slight changes in hematology parameters including increased eosinophils and basophils, and decreased lymphocytes, monocytes, white blood cells, platelets, and RBC. Clinical chemistry parameters such as AST and ALT were transiently increased at Days 90 and 120 while creatine and alkaline phosphatase were mildly decreased between 90 and 180 days post-administration. Post-mortem analysis showed no scAAV9.CBA.SMN -related inflammation in the liver, muscles, or heart. The NOAEL for this study was 3.3×10^{14} vg/kg.

Reviewer Comment:

This study used a scAAV9.CBA.SMN vector lot that was manufactured using a different process than that used for AVXS-101 clinical lots for this BLA. The concentration of the AVXS-101 vector lot used in this study relative to that of the AVXS-101 clinical vector is unknown. Similar to Study #13, the results of this toxicology study were different from the findings in the single IV administration of the of AVXS-101 in GLP studies #20122446 and #8384031, which showed heart and liver-targeted toxicities at dose levels of 7.9×10^{13} vg/kg and higher. The findings of this study cannot be directly used to support this BLA given the uncertainties in vector concentrations.

Study #15: Analysis of Vector Safety Following a Single Systemic Delivery of scAAV9.CBA.SMN into (b) (4) Macaques (NCH Safety analysis of scAAV9.CBA.SMN in NHP, Module 4.2.3.1)

Synopsis:

- This study evaluated the toxicology and transgene expression profiles following single IV administration of 6.7×10^{13} vg/kg scAAV9.CBA.SMN in 3-month old (b) (4) macaques for a period of 180 days. In-life and post-mortem evaluation revealed no test article-related adverse effects. Human SMN expression was present in all tissues with the highest levels in the adrenal gland, heart, liver, and skeletal muscles ($>1,000$ transcripts/ μ g mRNA). Lower expression was measured in the testes, brain, spinal cord, kidney, lung and intestines ($<1,000$ transcripts/ μ g mRNA). The NOAEL for this study was 6.7×10^{13} vg/kg AVXS-101.

Reviewer Comment:

The concentration of the nonclinical scAAV9.CBA.SMN vector lot used cannot be verified and therefore no conclusion can be drawn from this study regarding the safety for the dose level evaluated.

APPLICANT'S PROPOSED LABEL

Section 8 ('Use in Specific Populations) should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), 201.57(c)(14).⁵

Section 12.3 ('Pharmacokinetics') should be revised to accurately reflect the available data.

Section 13 ('Nonclinical Toxicology') should be revised to accurately reflect the available nonclinical data.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be adequately addressed in labeling. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

AAV, AAV9, scAAV9, SMA, SMN, gene therapy, ZOLGENSMA, AVXS-101, scAAV9.CBA.SMN, spinal muscular atrophy, survival motor neuron, SMNdelta7 mice, SMA mouse, (b) (4) macaque, nonhuman primate

⁵ Pregnancy and Lactation Labeling Rule (PLLR)