

# **CBER CMC BLA Review Memorandum**

**BLA STN 125694**

**onasemnogene abeparvovec-xioi  
ZOLGENSMA**

## **Reviewers**

Andrew Byrnes, Ph.D., OTAT/DCGT  
Angela Whatley, Ph.D., OTAT/DCGT

1. **BLA#:** STN 125694

2. **APPLICANT NAME**

AveXis, Inc.

3. **PRODUCT NAME/PRODUCT TYPE**

onasemnogene abeparvovec-xioi  
ZOLGENSMA

4. **GENERAL DESCRIPTION OF THE FINAL PRODUCT**

Pharmacological category: Adeno-associated virus vector-based gene therapy  
Dosage form: Suspension for injection  
Strength/Potency:  $2.0 \times 10^{13}$  vector genomes (vg) / mL  
Route of administration: Intravenous  
Indication: For 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

5. **MAJOR MILESTONES**

Received: October 1, 2018  
Filed: November 28, 2018  
Mid-cycle communication: January 29, 2019  
Late-cycle meeting: March 28, 2019  
PDUFA action due: May 31, 2019

6. **CMC/QUALITY REVIEW TEAM**

Reviewer/Affiliation	Section/Subject Matter
Andrew Byrnes, PhD, OTAT/DCGT/GTIB	As indicated in the review
Angela Whatley, PhD, OTAT/DCGT/GTB	As indicated in the review
Suzanne Epstein, PhD, OTAT/DCGT/GTIB	CBER consult review for MF (b) (4)

7. **INTER-CENTER CONSULTS REQUESTED**

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations (Yes/No)
Sharadrao Patil and Kang Chen Office of Testing and Research Office of Pharmaceutical Quality CDER	Validation of the analytical (b) (4) assay (in Section 3.2.P.5.3)	Yes
Kimberly Rains Office of Biotechnology Products Office of Pharmaceutical Quality CDER	Microbiological safety of (b) (4) (DMF (b) (4))	CDER review requested but was not provided. The DMF was reviewed by AW.

## 8. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
10/3/18	125694/0	Original submission
10/22/18	125694/5	Response to CM IR #3
11/20/18	125694/7	Response to CMC IR #5
11/20/18	125694/9	Response to CMC IR #7
11/20/18	125694/10	Response to CMC/Clinical IR #8
11/21/18	125694/11	Response to CMC IR #11
11/26/18	125694/12	Response to CMC IR #12
11/30/18	125694/16	Response to CMC IR #13
12/21/18	125694/18	Response to CMC IR #15
1/16/19	125694/20	Response to CMC IR #26
1/17/19	125694/21	Response to 11/28/18 filing letter
1/25/19	125694/26	Response to CMC IR #20
1/28/19	125694/28	Response to CMC IR #30
2/15/19	125694/34	Response to CMC IR #19
2/19/19	125694/36	Response to CMC IR #21
2/25/19	125694/37	Response to CMC IR #32
2/25/19	125694/38	Response to CMC IR #23
2/26/19	125694/41	Response to CMC IR #17
2/26/19	125694/42	Response to CMC IR #36
2/28/19	125694/44	Response to CMC IR #36
3/12/19	125694/45	Response to CMC IR #38
3/15/19	125694/46	Response to CMC IR #41
3/15/19	125694/47	Updated CMC modules
3/20/19	125694/48	Response to (b) (4) 483 observations
3/22/19	125694/50	Response to CMC IR #39
3/22/19	125694/51	Response to CMC IR #44
3/26/19	125694/52	Response to CMC IR #42
3/29/19	125694/53	Response to 1/29/19 Mid-Cycle Communication
4/9/19	125694/57	Response to CMC IR #47
4/10/19	125694/58	Response to CMC IR #46
4/11/19	125694/60	Response to 3/28/19 Late-Cycle Meeting
4/16/19	125694/62	Response to CMC IR #50
4/16/19	125694/63	Response to 3/28/19 Late-Cycle Meeting
4/17/19	125694/64	Response to CMC IR #48
4/19/19	125694/65	Response to CMC IR #52
4/22/19	125694/66	Response to CMC IR #51
4/26/19	125694/69	Response to CMC IR #53
5/2/19	125694/72	Response to CMC IR #55
5/6/19	125694/73	Response to CMC IR #54
5/6/19	125694/74	Response to CMC IR #57
5/7/19	125694/75	Updated CMC module
5/10/19	125694/79	Response to CMC IR #61
5/13/19	125694/80	Response to CMC IR #62
5/15/19	125694/81	Response to 5/8/19 teleconference
5/15/19	125694/83	Carton artwork update
5/16/19	125694/85	Response to CMC IR #65
5/20/19	125694/89	Updated labels

## 9. REFERENCED REGULATORY SUBMISSIONS (e.g., IND, BLA, 510K, MASTER FILE, etc.)

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Consult review: acceptable
DMF (b) (4)	(b) (4)	(b) (4)	Yes	Review not needed (MF (b) (4) contains sufficient information)
DMF (b) (4)	(b) (4)	(b) (4)	Yes	Review not needed (container chemical composition)
DMF (b) (4)	(b) (4)	(b) (4)	Yes	Review not needed (container chemical composition)
DMF (b) (4)	(b) (4)	(b) (4)	Yes	Review not needed (container chemical composition)
DMF (b) (4)	(b) (4)	(b) (4)	Yes	Review requested from CDER but not provided. Reviewed by AW.
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Review deferred to DMPQ
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Review deferred to DMPQ
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Review deferred to DMPQ

## 10. REVIEWER SUMMARY AND RECOMMENDATION

### A. EXECUTIVE SUMMARY

The CMC review team concludes that the manufacturing process for onasemnogene abeparvovec-xioi is capable of yielding a product with consistent quality characteristics, and the CMC review team recommends approval.

Onasemnogene abeparvovec-xioi is a suspension of an adeno-associated viral (AAV) vector-based gene therapy for intravenous infusion. The active ingredient is a recombinant self-complementary vector, where the double-stranded DNA vector genome is enclosed in a capsid that consists of (b) (4) 9 AAV capsid proteins. The vector (b) (4). The vector DNA contains a transgene encoding the human survival motor neuron (SMN) protein, under the control of a cytomegalovirus enhancer/chicken- $\beta$ -actin-hybrid promoter.

The drug product has a nominal concentration of  $2.0 \times 10^{13}$  vector genomes (vg)/mL. Each 10 mL vial of drug product contains an extractable volume of not less than either 5.5 mL or 8.3 mL and the excipients 20 mM tris (pH 8.0), 1 mM magnesium chloride ( $\text{MgCl}_2$ ), 200 mM sodium chloride (NaCl) and 0.005% poloxamer 188. The drug product is sterile and contains no preservative. The secondary packaging is a carton that contains 2-9 vials (depending on the weight of the patient) along with one alcohol wipe per vial. The carton is shipped frozen, and after receipt the carton should be stored in a refrigerator for no more than 14 days.

### *Manufacturing and quality*

(b) (4)

(b) (4) drug product are tested for general properties, including appearance, pH, osmolality and the molecular weights of AAV capsid proteins. The strength of (b) (4) drug product is measured by (b) (4), and strength is expressed in units of vg/mL.

The drug product is manufactured by (b) (4), and performing a sterile filtration. The drug product manufacturing process does not introduce any process-related impurities, and does not include any manufacturing steps that further remove impurities. (b) (4) drug product is filled aseptically into vials and frozen.

Drug product sampled from final containers is tested for microbial contaminants, identity, purity, strength and potency. Self-complementary AAV vectors such as onasemnogene abeparvovec-xioi have (b) (4)

(b) (4) in onasemnogene abeparvovec-xioi have been demonstrated to contain (b) (4)

(b) (4) The drug product specifications control the amounts of the various (b) (4) using an assay that quantifies the (b) (4).

The activity and potency of drug product lots are controlled using several assays, including a quantitative assay that measures the ability of the drug product to produce SMN protein in cells; a quantitative assay that measures (b) (4); and a semi-quantitative assay that measures the ability of intravenously-injected drug product to prolong mouse survival in a transgenic mouse model of spinal muscular atrophy.

### *Stability*

The drug product is stable for 14 days at refrigerated temperature, and 8 hours in syringes at room temperature. The drug product is not light-sensitive.

At the time of BLA submission, drug product stability had not been followed for a sufficient length of time to support the stability of drug product when stored at the long-term frozen storage

temperature of  $\leq -60^{\circ}\text{C}$ . Late in the review cycle, the applicant submitted real-time stability data from (b) (4) lots of drug product that had been stored for up to 1 year at the long-term frozen storage temperature. These data demonstrate time-dependent declines in the strength, activity and potency of onasemnogene abeparvovec-xioi. When stored frozen at the long-term storage temperature, the strength (vector genome concentration) declines at a rate of approximately (b) (4) over the first year, with significant uncertainty about the rate of decline in subsequent years. As a result, the shelf life of DP stored at the long-term frozen temperature will be limited to 12 months.

The completed phase 1 clinical trial (NCT02122952) was conducted using a single lot of onasemnogene abeparvovec-xioi drug product, administered to two cohorts of subjects. The doses administered in this phase 1 study were originally reported to be  $6.7 \times 10^{13}$  vg/kg and  $2.0 \times 10^{14}$  vg/kg, but the assay that was originally used to determine the concentration of this initial drug product lot was inaccurate and imprecise. Forty-four months after manufacture of this initial drug product lot, the vector genome concentration was revised based on measurement with an accurate and precise assay. Based on the revised concentration of the initial drug product lot, the doses in the phase 1 study were retrospectively restated as  $3.7 \times 10^{13}$  vg/kg and  $1.1 \times 10^{14}$  vg/kg.

Stability data submitted late in the review cycle indicate that onasemnogene abeparvovec-xioi is unstable during long-term frozen storage. Because of uncertainty about the rate of decay of the initial drug product lot, the Agency is unable to determine the doses that were administered to subjects in the phase 1 trial. The Agency estimates that the doses administered in cohort 1 of the phase 1 trial may have ranged from  $4.3 \times 10^{13}$  to  $4.6 \times 10^{13}$  vg/kg, and the doses administered in cohort 2 may have ranged from  $1.1 \times 10^{14}$  to  $1.4 \times 10^{14}$  vg/kg, with considerable uncertainty.

Newly-manufactured lots of drug product were used in all ongoing clinical trials with onasemnogene abeparvovec-xioi, and the vector genome concentrations of these lots were determined using an accurate and precise assay. The  $1.1 \times 10^{14}$  vg/kg dose that was used in ongoing clinical trials is accurate.

#### *Comparability*

After the phase 1 clinical trial using the initial clinical lot, the manufacturing process was changed considerably. The current manufacturing process produces drug product with critical quality attributes that are comparable to those of the initial clinical lot. Although the concentration of drug product declines over time during storage, the ratio of potency to vector genomes is comparable when lots from the current manufacturing process are compared directly to the initial clinical lot, including comparable ability to enhance survival in a mouse model of SMA. Drug product manufactured using the current manufacturing process has better purity (b) (4)

## **B. RECOMMENDATION APPROVAL**

This biological license application (BLA) provides an adequate description of the manufacturing process and characterization of the new drug product onasemnogene abeparvovec-xioi. The CMC review team has concluded that the manufacturing process, along with associated test methods and control measures, is capable of yielding a product with consistent quality characteristics. This information, along with post-marketing commitments (PMC) from AveXis, Inc., satisfies the CMC requirements for biological product licensure per the provisions of section 351(a) of the Public Health Service (PHS) Act controlling the manufacture and sale of biological products.

#### *Lot release*

This product is subject to CBER lot release, and the lot release protocol is provided in the DBSQC review memo.

*Post-marketing commitments (PMC #1 in amendment 85 on May 16, 2019, and PMCs #2 and #3 in amendment 74, May 6, 2019):*

1. AveXis agrees to develop and qualify a suitable method for quantifying (b) (4) providing the method qualification report and providing an additional process validation report for (b) (4) by 31 December 2019.
2. AveXis agrees to validate the robustness of the (b) (4) assay per protocol REC-2566 and will provide the validation report by 31 December 2019.
3. AveXis agrees to update the (b) (4) assay to include the assay validity criterion for the reference standard and provide the supplemental validation report for robustness by 31 December 2019.

#### **SIGNATURE BLOCK**

<b>Reviewer/Title/Affiliation</b>	<b>Concurrence</b>	<b>Signature and Date</b>
<b>Angela Whatley, Ph.D.</b> Biologist DCGT/OTAT	Concur	
<b>Andrew Byrnes, Ph.D.</b> Supervisory Research Microbiologist DCGT/OTAT	Concur	
<b>Denise Gavin, Ph.D.</b> Chief, Gene Transfer Branch DCGT/OTAT	Concur	
<b>Raj Puri, M.D., Ph.D.</b> Director, Division of Cellular and Gene Therapies OTAT	Concur	

## **Review of CTD**

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## Module 3

### 3.2.S DRUG SUBSTANCE

#### 3.2.S.1.1 - 1.3 Nomenclature, Structure and General Properties (reviewed by AW)

##### 3.2.S.1.1 Nomenclature

Proper (non-proprietary) name: onasemnogene abeparvovec-xioi

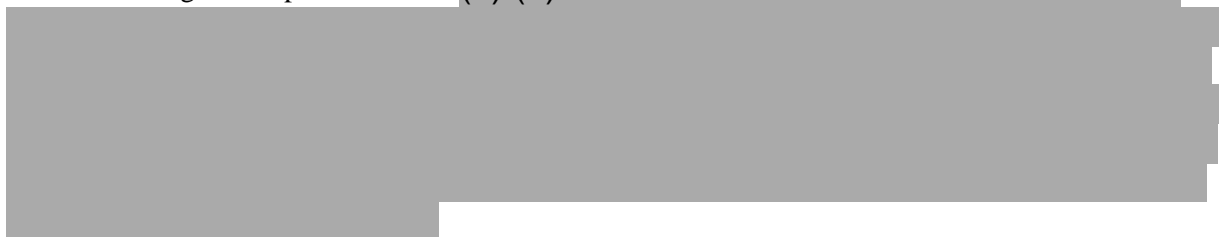
Proprietary name: ZOLGENSMA

*Table 1 Nomenclature*

International Nonproprietary Name (INN)	onasemnogene abeparvovec
United States Adopted Name (USAN)	onasemnogene abeparvovec
Company or Laboratory Code(s)	AVXS-101 (previously termed sc.AAV9.CB.SMN)
Chemical Abstracts Service Registry Number	(b) (4)
Chemical Abstract Service Index Name	DNA (synthetic adeno-associated virus 9 vector scAAV9.CB.hSMN human survival motor neuron protein-specifying)

##### 3.2.S.1.2 Structure

The onasemnogene abeparvovec- xioi (b) (4)



(b) (4)

(b) (4)

(b) (4)



- (b) (4)

(b) (4)

### Development of the Process Control Strategy (reviewed by AW)

Development of the process control strategy began with defining the Quality Target Product Profile (QTPP). The QTPP was then used to evaluate potential CQAs and decide which were critical and which were non-critical. A risk assessment was also done to evaluate the risk of each process parameter to impact CQAs. The design space for a subset of critical process parameters was evaluated with small scale studies done with a (b) (4) process. Finally, Process Performance Qualification (PPQ) runs were conducted to demonstrate that the applicant can consistently manufacture within the predefined operating parameters. The major steps in this process are reviewed in this section below.

The process control strategy involved determining the quality target product profile, which informed the CQA selection. The AVXS-101 Quality Target Product Profile served as a basis for development of the manufacturing process and describes the high-level quality, safety and efficacy requirements for AVXS-101. Among other key attributes, the route of administration, dosage form, strength, and stability targets for AVXS-101 are defined in Table 11 AVXS-101 Drug Product Quality Target Product Profile.

*Table 11 AVXS-101 Drug Product Quality Target Product Profile*

Product QTPP Element	Product QTPP Element Target	Justification
Indications and Usage	AVXS-101 is an adeno-associated virus (AAV) vector-based gene therapy indicated for the treatment of pediatric patients with spinal muscular atrophy (SMA).	Efficacy claim for AVXS-101.

Route of Administration	AVXS-101 (IV) is administered as a slow intravenous infusion over approximately 60 minutes.	Route of administration for AVXS-101 used in clinical studies.
Patient Population	Intravenous administration is intended for pediatric patients between (b) (4) and 8.5 kg with spinal muscular atrophy.	Efficacy claim for AVXS-101.
Contraindications.	None.	None are known.
Drug Interactions	No drug-drug interaction studies have been conducted.	Not determined to be necessary based on patient population.
Concentration	AVXS-101 drug product for intravenous administration should be formulated at a target concentration of $2.0 \times 10^{13}$ vg/mL.	Target concentration based on pharmaceutical development and intended doses.
Excipients	Each (b) (4) of AVXS-101 (IV) DP solution in (b) (4) contains 20 mM Tromethamine, 1 mM Magnesium Chloride, 200 mM Sodium Chloride, and 0.005% m/V Poloxamer 188 ((b) (4)).	(b) (4)
Dosage Form and Volume	intravenous infusion in pediatric patients. The recommended dose of AVXS-101 for intravenous infusion in pediatric patients with a body weight of (b) (4) to 8.5kg is $1.1 \times 10^{14}$ vector genomes/kg.	Ease of administration, stability of product during administration and transport, compatibility with desired product efficacy, and volumes necessary to meet recommended dosage.
Dosage Strength	The intravenous dosage strength studied in clinical trials was (b) (4). The planned commercial intravenous dosage strength is $2.0 \times 10^{13}$ vg/mL.	Recommended dosage based on clinical trial data.
Container Closure System	AVXS-101 is supplied in (b) (4), cyclic olefin polymer 10mL vials. The vials are stoppered with a 20 mm Chlorobutyl rubber serum stopper with silicone coating, the vials are finally sealed with an aluminum seal and plastic flip cap.	Recommended storage using commonly available container closure components. Non-glass is preferred to avoid breakage and assure seal integrity at cryo temperatures.
Delivery System	When preparing to dose a patient, AVXS-101 product will be shipped frozen ( $\leq -60^{\circ}\text{C}$ [ $-76^{\circ}\text{F}$ ]) to the healthcare site. Product must be thawed before preparation and administration to the patient. A healthcare professional (HCP) will then transfer the AVXS-101 product from each of the vials packaged in the SKU into a syringe. When the entirety of the product required for dosing is pooled, the syringe is capped and delivered to the treatment location.	Recommended delivery system based on clinical trial study design.







- (b) (4)

### 3.2.P DRUG PRODUCT

### 3.2.P.1 Description and Composition of the Drug Product (reviewed by AW)

AVXS-101 Drug Product (DP) is a single-dose, preservative-free, sterile, clear to slightly opaque, and colorless to faint white, intravenous infusion of non-replicating, self-complementary AAV9 vector at a target concentration of  $2.0 \times 10^{13}$  vg/mL. Each (b) (4) of AVXS-101 DP solution in (b) (4) contains 20 mM Tromethamine (Tris), (b) (4) Magnesium Chloride, 200 mM Sodium Chloride, and 0.005% w/v Poloxamer 188. The pH range of the solution is (b) (4).

Component	Quality Standard	Function	Quantity per mL	Quantity per 5.5 mL vial	Quantity per 8.3 mL vial
AVXS-101 (b) (4)	In-House Standard	Active Ingredient	(b) (4)	(b) (4)	(4)
Tromethamine	(b) (4)	(b) (4)			
Magnesium Chloride	(b) (4)	(b) (4)			
Sodium Chloride	(b) (4)	(b) (4)			
Poloxamer 188	(b) (4)	(b) (4)			
(b) (4)	(b) (4)	(b) (4)			
(b) (4)	(b) (4)	(b) (4)			

(b) (4)

AVXS-101 DP is filled into 10 mL (b) (4) vials with a nominal fill volume of 5.5 mL or 8.3 mL and stored at  $\leq -60^{\circ}\text{C}$ . Each vial also includes a target (b) (4).


AVXS-101 DP is filled in a sterile, ready to use, 10 mL, (b) (4) vial. The vial is sealed with a sterile, ready to use, 20 mm, (b) (4) Gray, chlorobutyl elastomeric stopper. The stopper is capped with a sterile, 20 mm flip-off, aluminum seal with a colored plastic button cap.

### 3.2.P.2 Pharmaceutical Development


#### 3.2.P.2.1 Components of the Drug Product

##### 3.2.P.2.1.1 (b) (4) (reviewed by AW)

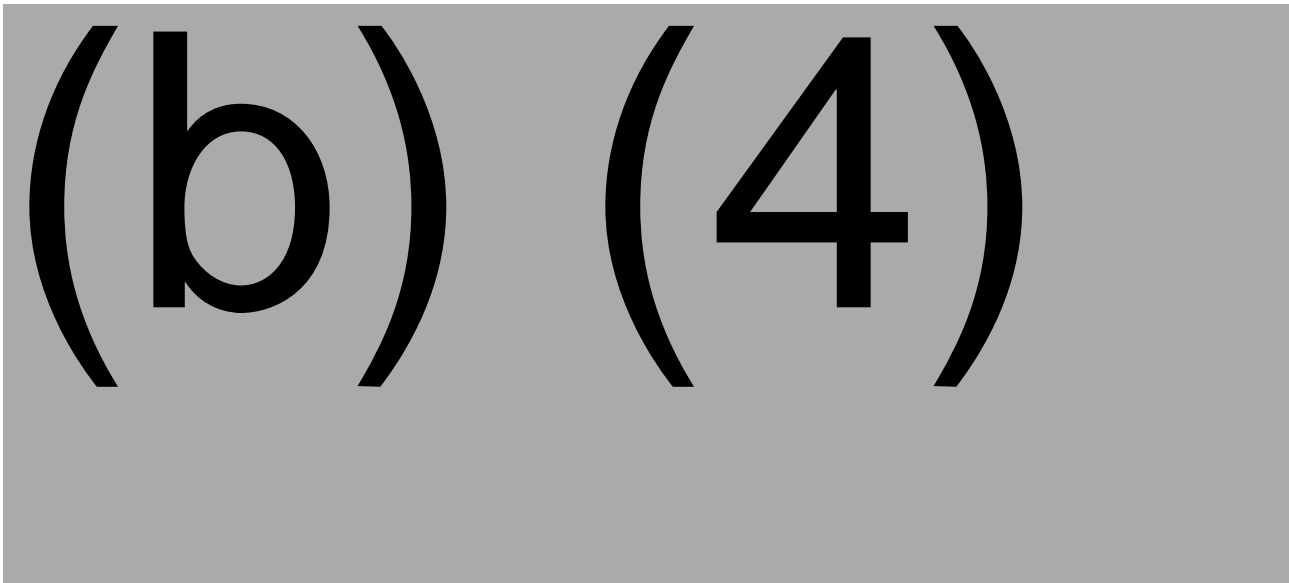
(b) (4)




(b) (4)



(b) (4)



(b) (4)



### 3.2.P.2.2 Drug Product

#### 3.2.P.2.2.1 Formulation Development (Reviewed by AB)

DP is formulated at a concentration of  $2.0 \times 10^{13}$  vg/mL in a (b) (4) that is composed of standard (b) (4) excipients. The density is (b) (4), and this density value is used when calculating the fill volume controls.

It is commonly thought that pH, high salt and surfactant may be critical factors for the stability of AAV vectors (b) (4). The impact of pH (b) (4) on AAV9 stability is not specifically known. Studies by AveXis (when optimizing their (b) (4) assay) show that the presence of surfactant is critical for preventing (b) (4) of AAV9 onto plastics and other surfaces.

Magnesium has been reported to (b) (4) of a non-AAV parvovirus, but the effect on AAV9 has not been specifically evaluated.

The initial DP lot used in CL-101 had a slightly different formulation. Lot AAV9SMN0613 was formulated by NCH with (b) (4), rather than (b) (4). The initial (b) (4) AveXis lots (816836 and 816841) were also formulated with (b) (4).

Except for the change in (b) (4), the applicant did not report any formulation development studies.

Mouse tox studies were performed with lot (b) (4) and 600443 (b) (4). Lot 816836 was administered to two subjects in study CL-303, but all other subjects in CL-303 received product in the (b) (4) formulation.

*Reviewer comments: Because the first (b) (4) lot was manufactured only in (b) (4), the BLA was submitted with only a limited duration of stability information in the (b) (4) formulation, with most of the information delayed until March 29, 2019 in amendment 53. This late stability submission indicates that the vg concentrations of lot AAV9SMN0613 (b) (4) and the AveXis lots are both declining in vg concentration during storage. AveXis lots 816831 and 816841 (b) (4) were not evaluated for stability.*

*A number of the assay validations were performed using the (b) (4) formulation, and this is noted in the review of each such assay validation. The applicant evaluated compatibility of DP with delivery devices using only the (b) (4) formulation (3.2.P.2.6), but there were no concerns, and the compatibility in the (b) (4) formulation should be similar to or better than compatibility in the (b) (4) formulation.*

#### 3.2.P.2.2.2 (b) (4)

The original submission did not propose an (b) (4). However, the stability data in amendment 53 raised the concern that the DP vg concentration might not remain within an acceptable range during the entire shelf life. During a teleconference on May 2, 2019, the applicant proposed a small (b) (4) and FDA agreed with this plan. For new lots of DP manufactured under the license, the target concentration will be (b) (4) vg/mL, instead of the nominal concentration of  $2.0 \times 10^{13}$  vg/mL. The manufacturing process description in module 3.2.P.3.3 was updated in amendment 75 (May 7, 2019) to reflect the new target concentration of (b) (4) vg/mL.

#### 3.2.P.2.2.3 Physicochemical and Biological Properties

(b) (4) DP have the same formulation and same properties, except that the (b) (4) than in (b) (4).

#### 3.2.P.2.3 Manufacturing Process Development

**Process development** (reviewed by AB)

Table 25 summarizes the changes in DP manufacturing. Notable differences include:

- There was an (b) (4) (in the NCH lot and the first (b) (4) AveXis lots) to (b) (4) (in all other AveXis lots). Other than the (b) (4), the formulation and pH have remained constant.
- Change in final container from (b) (4) vials (for all AveXis lots). The 10 mL vial size for the PPQ lots (b) (4) is the same 10 mL vial as for the proposed commercial process.
- The concentration of NCH lot AAV9SMN0613 was measured at (b) (4) vg/mL using the AveXis (b) (4) in August, 2017, but because of instability it is unclear whether this value adequately represents the concentration of AAV9SMN0613 at the time that this lot was administered to subjects in study CL-101. A subset of the AveXis lots were manufactured at the proposed commercial target concentration of  $2.0 \times 10^{13}$  vg/mL, including the (b) (4) PPQ lots. Other lots were filled at (b) (4) vg/mL – there is an ongoing clinical trial with (b) (4) administration of the product, which requires a high product concentration.
- (b) (4) was performed at NCH using a (b) (4). The AveXis (b) (4), which has the potential to improve purity.
- Filling of AAV9SMN0613 at NCH was a manual process. Filling at AveXis is automated.
- The applicant changed the fill volumes frequently during development, and the AveXis PPQ lots were filled at a slightly greater volume (b) (4) than the intended commercial fill volume (5.5 and 8.3 mL). The applicant has separate MBRs for each of the fill volumes.
- Storage temperature has remained constant at  $\leq -60^{\circ}\text{C}$ .
- The analytical methods have been completely redeveloped by AveXis. When possible, the applicant used the new AveXis methods to evaluate lot AAV9SMN0613 and to determine comparability. The only exception is the AveXis (b) (4) assay, which could not be used to assay lot AAV9SMN0613 because it does not detect the (b) (4) used in the NCH manufacturing process (the NCH (b) (4) that are detected in the AveXis assay).

Process Attribute	Process A	Process B - Initial	Process B – Proposed Commercial
Manufacturing Site	Nationwide Children's Hospital, Columbus, Ohio	AveXis, (b) (4)	
Use	Phase 1 Clinical	Pivotal Clinical Trial and Development	Proposed Commercial <sup>1</sup>
Lots	AAV9SMN0613	816836 (b) (4) 600307 600443 600156	(b) (4)
Formulation Composition	(b) (4)		
	20 mM Tromethamine (Tris)		
	1 mM Magnesium Chloride		
	200 mM Sodium Chloride		
	(b) (4) w/v Poloxamer 188	(b) (4) and 0.005% <sup>3</sup> w/v Poloxamer 188	0.005% w/v Poloxamer 188
	pH range of the solution is (b) (4)		
(b) (4)	(b) (4)		
Fill Date	(b) (4)		
DP Concentration	1.1 x 10 <sup>13</sup> vg/mL <sup>4</sup>	3.7 x 10 <sup>13</sup> to 5.3 x 10 <sup>13</sup> vg/mL	(b) (4) to 2.0 x 10 <sup>13</sup> vg/mL
Container Closure System	2 mL and 5 mL (b) (4)	5 mL (b) (4) Cyclic Olefin	10 mL (b) (4) Cyclic Olefin Polymer Vial; 20 mm Stopper; 20 mm Seal
Fill Volume	(b) (4)	(b) (4)	Nominal: (b) (4) 5.5 mL and 8.3 mL
Process Steps	<ul style="list-style-type: none"> <li>• (b) (4) Formulation (b) (4)</li> <li>• Sterile Filtration</li> <li>• Filling</li> <li>• Labeling</li> <li>• Secondary Packaging</li> </ul>	<ul style="list-style-type: none"> <li>• Sterile Filtration &amp; (b) (4)</li> <li>• Filling</li> <li>• Visual Inspection</li> <li>• Labeling</li> <li>• Secondary Packaging</li> </ul>	<ul style="list-style-type: none"> <li>• (b) (4)</li> <li>• Filling</li> <li>• Visual Inspection</li> <li>• Labeling</li> <li>• Secondary Packaging</li> </ul>

(b) (4) DP = Drug Product; DS = Drug Substance; (b) (4)

<sup>1</sup> These lots used the proposed commercial process; however, are not designated for commercial distribution. They may be designated for clinical use, as needed.

<sup>2</sup> Lot not released, used for development purposes only.

<sup>3</sup> Implemented as part of Lot (b) (4)

(b) (4)

Table 25 Development of the DP manufacturing process

*Reviewer comments: The major process differences are between lot AAV9SMN0613 (process A) and the AveXis lots (process B). Among the AveXis lots, one major change is in the (b) (4), which changed from (b) (4) (2 subjects in CL-303 received 816836, and no subjects received 816841) to (b) (4) (all other subjects in CL-303). Another major change is the product concentration, which was changed to 2.0×10<sup>13</sup> vg/mL. All of the PPQ lots were formulated at this product concentration, which is the same as the commercial concentration. All subjects in study CL-303 received DP manufactured by initial process B, except for one subject who received lot 600629 (PPQ lot, process B - commercial). The changes between process B-initial and B-commercial (b) (4), product concentration, fill volume) are unlikely to affect the quality of the product, and analysis of the product quality attributes does not give any indication for concern.*

*The second of the PPQ lots (b) (4) was (b) (4) concentration and sterile filtration) because the concentration of this lot did not initially meet the target range. The (b) (4) PPQ lot (b) (4) was (b) (4) due to a leak during the initial sterile filtration that might have compromised sterility. Neither (b) (4) were administered to subjects in CL-303.*

*The (b) (4) issue was discussed during the (b) (4) inspection and the March 28, 2019 late-cycle meeting – we will allow (b) (4) in cases of mechanical/equipment failure, but will not allow (b) (4) to be part of the standard manufacturing procedures unless the applicant provides adequate evidence in a PAS that there is no negative impact on the product.*

*The changes in the fill volumes during development of the AveXis process are trivial, and the PPQ lots were filled at volumes that are only (b) (4) than the final 5.5 mL and 8.3 mL fill volumes. Appropriate studies were performed to verify that there is sufficient (b) (4) to allow full recovery of the labeled 5.5 mL and 8.3 mL volumes (3.2.P.2.3.4).*

#### **DP comparability**

The BLA included one formal comparability study (RPT-446) comparing lot AAV9SMN0613 to pre-PPQ lots 600156 and 600307. This study was previously submitted to IND 15699 in mid-2018 for discussion during the pre-BLA meeting, and FDA agreed at the time that the study provided evidence of analytical comparability between AAV9SMN0613 and the AveXis lots that was adequate for the purpose of allowing BLA submission (*i.e.*, not a refuse to file issue).

The PPQ report RPT-399 also contain extensive analysis of the (b) (4) PPQ lots at (b) (4) and the DP stage. Although this is not a formal comparability report, the data can be seen to be comparable to AAV9SMN0613 with the following exceptions (also analyzed elsewhere in this review):

- (b) (4)

Beyond the data in these reports, the BLA contains extensive information from additional AveXis lots, and this information continues to support consistency of AveXis lots and comparability of AveXis lots with AAV9SMN0613 (see 3.2.S.4.5 and 3.2.P.5.6 for graphs of lot release data from additional AveXis lots). In some cases (b) (4) the purity of the AveXis lots is substantially better than AAV9SMN0613.

As discussed in more depth below, mouse survival data from the old in vivo potency assay (SOP-285) provide evidence in favor of the comparability of biological activity between AAV9SMN0613 and AveXis lots. FDA's independent re-analysis of AveXis's historical mouse survival data from REC-1606 (amendment 3) supports comparability of in vivo potency between the AveXis lots and AAV9SMN0613, although the sensitivity of this analysis is somewhat limited.

#### Applicant's initial comparability study

The comparability study reported below (RPT-446) is located in 3.2.R and summarized in 3.2.P.2.3.3.3. (b) (4) AveXis pre-PPQ lots (b) (4) were compared to lot (b) (4). To allow equivalent comparisons among lots that have different vg concentrations, the applicant normalized quantitative criteria to (b) (4) vg. Qualitative attributes (appearance, (b) (4)) are clearly comparable (Table 26). Points of note regarding quantitative attributes:





Test	NCH AAV9SMN0613	AveXis 600156	AveXis 600307
(b) (4)			
pH	(b) (4)		
Osmolality (mOsm/kg)	(b) (4)		
Appearance by visual inspection	Clear and colorless solution, free of visible particles	Faint white, slightly opaque, free of visible particles	Colorless, slightly opaque, free of visible particles
Total protein	(b) (4)	(b) (4)	
(b) (4)			

Table 26 AveXis comparability study RPT-446

(b) (4)

(b) (4)

(b) (4)

(b) (4)

#### **Comparability of mouse survival data**

SMNΔ7 mice die at 2-3 weeks after birth if they are not treated with vector, and the length of survival depends on the dose of the vector. Under IND 15699, AveXis developed a complicated in vivo potency assay (SOP-285) to evaluate the activity of each new DP lot in multiple groups of neonatal mice at several different i.v. vector doses. Multiple assays were performed between early 2017 and early 2018, comparing survival with AAV9SMN0613 to survival with AveXis lots.

FDA disagreed with the method that AveXis was using to analyze the data in SOP-285. Although this potency assay has now been replaced by the new in vitro and in vivo potency assays (SOP-347 and SOP-346), the large amount of historical mouse survival data from SOP-285 can still be analyzed to evaluate comparability. The survival data were provided in REC-1606 (amendment 3). There had been a number of discrepancies in a previous report on this historical data (REC-1225, submitted to IND 15699), and REC-1606 contains audited data that has been corrected and verified by the applicant. During inspection of the AveXis (b) (4) facility, FDA also verified some of the data in REC-1606 by comparing to original records.

In the SOP-285 assay, groups of neonatal mice were injected with various doses of the reference article (NCH lot AAV9SMN0613) or the test article (AveXis lot). Control mice were uninjected or injected with formulation buffer. Any mice that died at 10 days or less were assumed to have died for unrelated reasons and were excluded (per protocol) from the assay analysis. The FDA plots shown below exclude these mice that died at  $\leq 10$  days. For most studies, the doses were  $1 \times 10^{12}$ ,  $1.2 \times 10^{13}$ ,  $7.5 \times 10^{13}$  and  $1.1 \times 10^{14}$  vg/kg. In some studies, the doses  $7.4 \times 10^{13}$  and  $2.9 \times 10^{14}$  vg/kg were used. The clinical dose is  $1.1 \times 10^{14}$  vg/kg.

In the following survival curve analyses (Figure 33), data from various assays are pooled to increase power (including pooling two slightly different doses:  $7.4 \times 10^{13}$  and  $7.5 \times 10^{13}$  vg/kg). The  $1 \times 10^{12}$  vg/kg

data are not shown below because this dose of vector did not increase survival. Note that some of the comparisons lack power because of very small group size (e.g.,  $n = 4$  for 816836 at  $1.1 \times 10^{14}$  vg/kg).

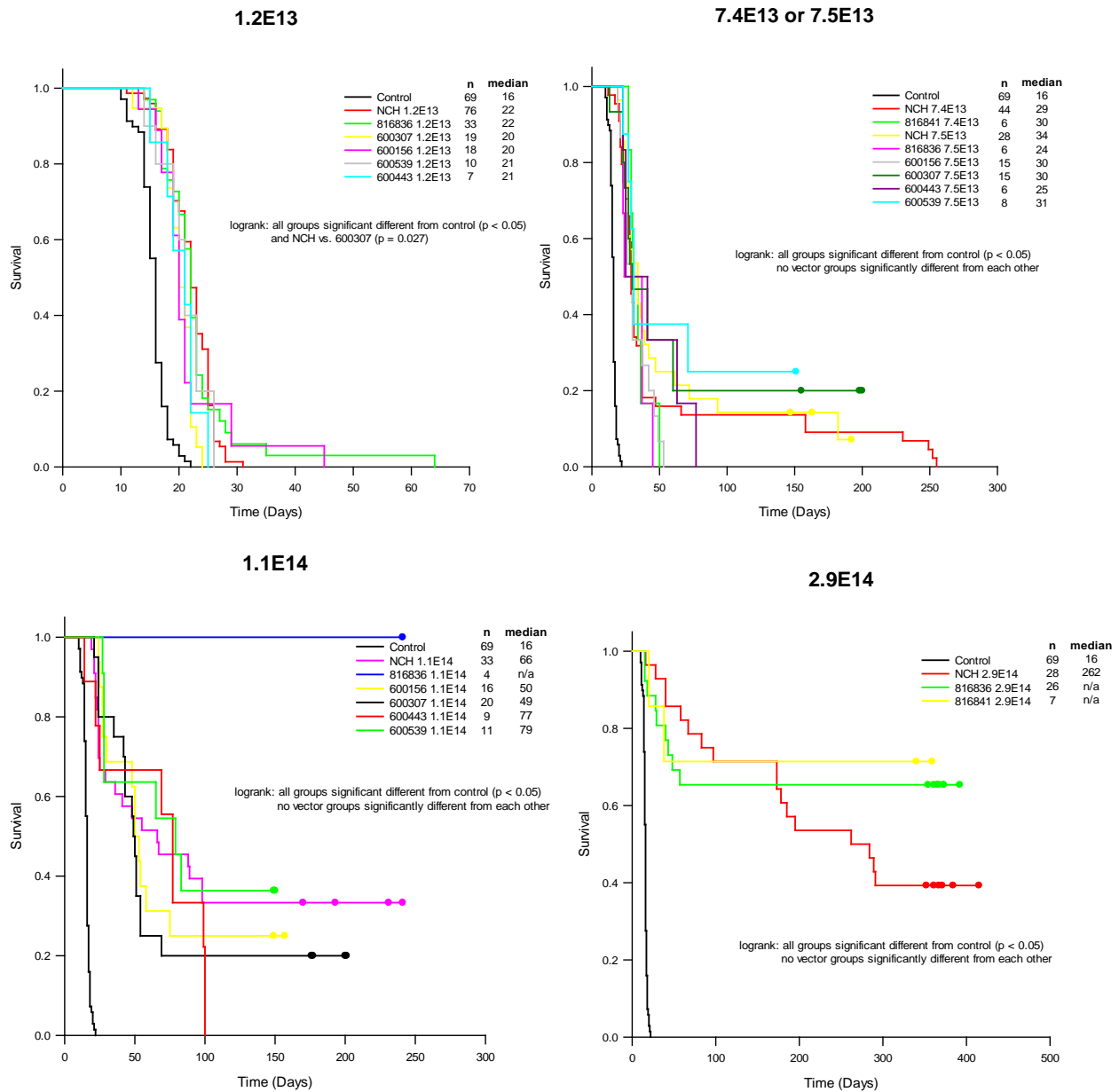


Figure 33 Survival analysis in neonatal SMN $\Delta$ 7 mice: comparison of NCH lot (AAV9SMN0613) to AveXis lots at various doses

These data indicate that all vector lots cause significantly increased survival of SMN $\Delta$ 7 mice at doses of  $1.2 \times 10^{13}$  vg/kg and above. None of the vector lots differ significantly from any of the other vector lots, except at  $1.2 \times 10^{13}$  vg/kg where mice treated with AAV9SMN0613 showed greater survival than mice treated with lot 600307 ( $p < 0.05$ ). This difference is likely a chance finding. The difference in median survival at  $1.2 \times 10^{13}$  vg/kg is very small, and there is no apparent difference in survival when comparing the NCH lot and 600307 at the  $7.5 \times 10^{13}$  and  $1.1 \times 10^{14}$  vg/kg doses. All available survival data for lot 600307 are further examined in Figure 34. The analysis across all lots is consistent with equal potency between the two lots.

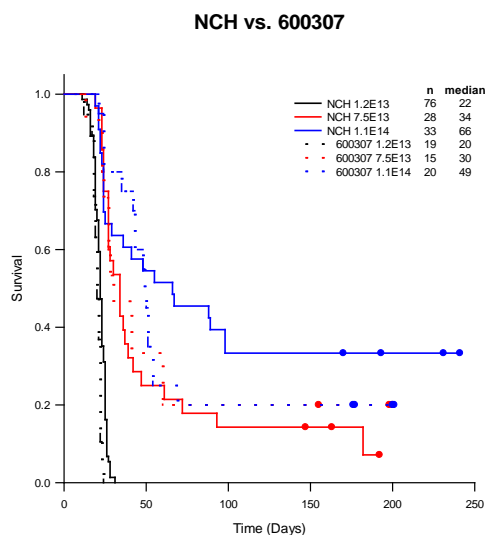
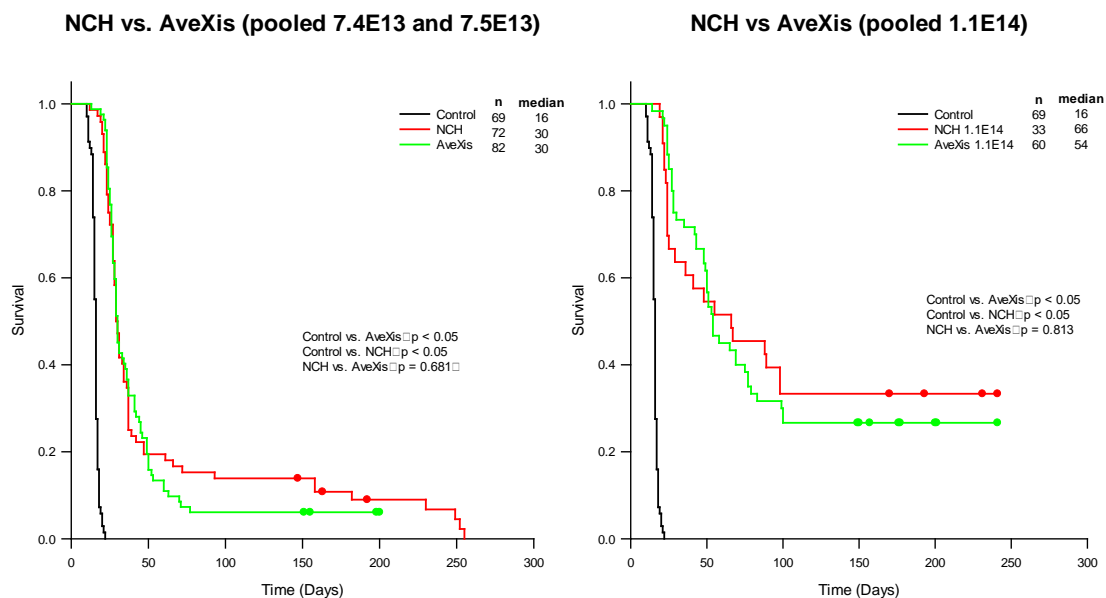


Figure 34 Survival analysis of AAV9SMN0613 vs. lot 600307

To examine potential differences in potency among lots that might be attributed to differences between the NCH manufacturing method and the AveXis manufacturing method, FDA pooled survival data from lots at  $7.4\text{--}7.5 \times 10^{13}$ ,  $1.1 \times 10^{14}$  and  $2.9 \times 10^{14}$  vg/kg. There were no significant differences in survival between NCH and AveXis lots (Figure 35).



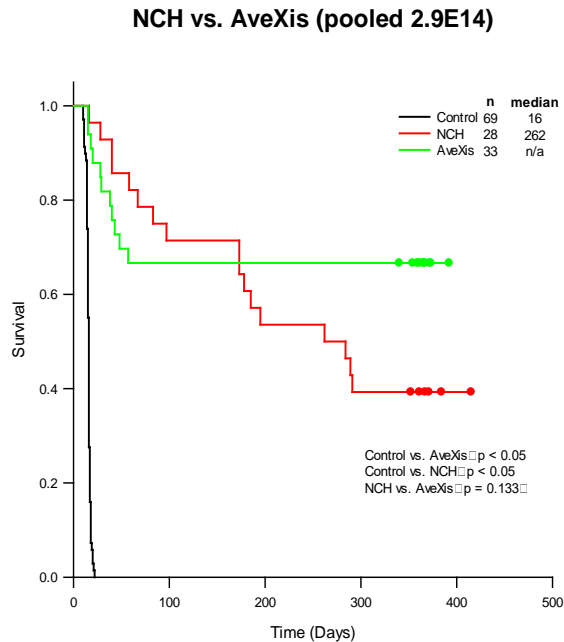


Figure 35 Survival analysis of AAV9SMN0613 vs. all pooled AveXis lots

The pooled survival data can also be compared between different doses, which helps to estimate the power of these types of analyses. When comparing between the  $7.4\text{--}7.5 \times 10^{13}$  and  $1.1 \times 10^{14}$  vg/kg groups (Figure 36, left), the decrease of about 33% in vector dose (from  $1.1 \times 10^{14}$  to  $7.5 \times 10^{13}$  vg/kg) is easily detectable as a decrease in survival, suggesting that this type of analysis would have detected a 33% difference in vector potency between the AveXis and NCH groups, if such a difference had existed. When comparing the  $1.1 \times 10^{14}$  and  $2.94 \times 10^{14}$  vg/kg groups (a 63% difference in vector dose), the differences in survival are marginally detectable. The analysis in the graph on the right likely has much lower power due to a smaller number of animals and greater amount of censoring (total of 247 mice with 35 censored for the left graph, as compared to 154 mice with 60 censored for the right graph).

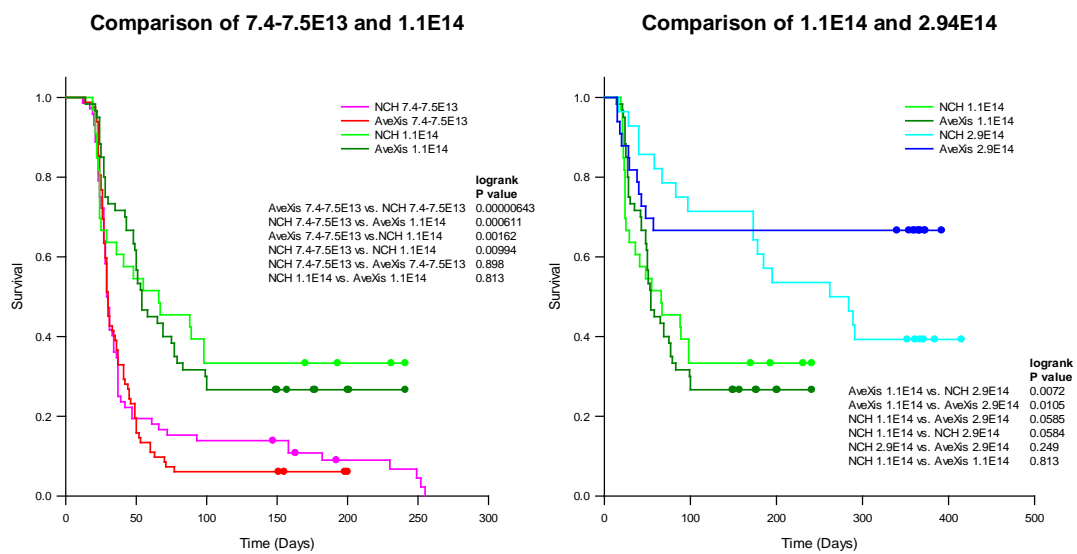


Figure 36 Dose-dependency of survival

The studies in REC-1606 were performed over an approximately one year period between March, 2017 and early 2018. The concentration of AAV9SMN0613 in all of the in vivo potency assays is based on the August, 2017 (b) (4) value of  $1.06 \times 10^{13}$  vg/mL (applied retroactively in the case of in vivo potency assays performed before August, 2017). This concentration is not reliable across time due to vector instability, but the amount of inaccuracy (likely no more than (b) (4) from early 2017 to early 2018) is insufficient to make a major impact on the analysis of the in vivo data above, which can detect (at best) a 30% difference in potency. Additional analysis of mouse survival with AAV9SMN0613 across the in vivo potency assays does not reveal a downward trend in survival with time (Figure 37), but this type of analysis of median survival has much more limited sensitivity than the logrank comparisons above.

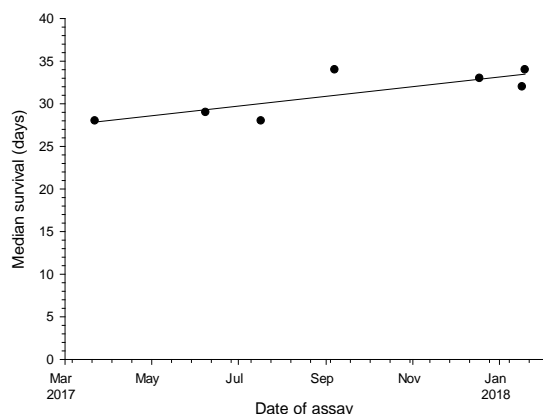


Figure 37 Median survival in seven in vivo potency assays with AAV9SMN0613 at a dose of  $7.4-7.5 \times 10^{13}$  vg/kg

*Reviewer comment: Together, all of these analyses of the survival data in REC-1606 support the idea that differences between the NCH and AveXis manufacturing processes likely do not cause any detectable differences in vector potency per unit of vg concentration in SMNΔ7 mice.*

#### *Impact of manufacturing process changes on potency*

In vitro potency data were submitted in amendment 53 in RPT-1015. These potency data are also analyzed in sections 3.2.S.4.5 and 3.2.P.5.6 of this review. As indicated in Table 27, there are no apparent differences in potency between the various AveXis lots.

*Reviewer comment: both potency assays are variable, and interpretation of the in vitro potency data is clouded by the instability of vg concentration and potency over time for DP lots and (we assume) for the reference standard vector RS-002.*

Table 27 Comparability of in vitro and in vivo potency

DP lot	DOM	In vitro potency (percent)	In vivo potency (median survival)*	Manufacturing process
AAV9SMN0613	(b) (4)	(b) (4)	(b) (4)	Process A
600156***				Process B initial
600307***				Process B initial
600443				Process B initial
600539				Process B PPQ
600482				Process B PPQ
600480				Process B PPQ

600629	(b) (4)	Process B PPQ
601002		Process B commercial
601006		Process B commercial
601010		Process B commercial
601071		Process B commercial
601182		Process B commercial
601183		Process B commercial
601436		Process B commercial
601121		Process B commercial
601122		Process B commercial
601120		Process B commercial

\* After finalization of the new in vivo potency assay, results are simply reported as >24 if more than half of the mice in the test article group survive for at least 24 days. This guarantees that the median will meet the specification, even if the actual median survival has not yet been reached.

\*\* In vitro potency of (b) (4) measured in May, 2018, and (b) (4) measured in March, 2019 (from RPT-1333 in amendment 60).

\*\*\* Lot administered in clinical trial CL-303.

*Reviewer comment: Evaluation of comparability is difficult due to changes in assays, variable assays, manufacturing problems with early AveXis lots and the instability of DP over time. Some conclusions can be reached, however:*

- The protein composition is equivalent between the AveXis lots and AAV9SMN0613.*
- The formulation is different ((b) (4)) for AAV9SMN0613, and ((b) (4)) for most AveXis lots), but there is no evidence and no expectation that this difference would alter the product CQAs.*
- In terms of purity, the post-PPQ AveXis lots are equivalent or superior to lot AAV9SMN0613.*
- The in ((b) (4)) assay indicates that potency per unit vg is similar among AveXis lots and as compared to lot AAV9SMN0613, with the caveat that this assay has moderate variability.*
- FDA reanalysis of the in vivo SOP-285 survival data provides support for comparability between vector manufactured by AveXis and lot AAV9SMN0613, with the caveat that this analysis has moderate power (it likely would have detected a difference in potency of 30%, if such a difference had existed).*
- The vg concentration of AAV9SMN0613 at the time of study CL-101 is currently unknown because of the instability of the vector, but the concentration in 2014 and 2015 was almost certainly higher than the ((b) (4)) vg/mL concentration measured by ((b) (4)) in August, 2017.*

### **Development of the Process Control Strategy (reviewed by AW)**

The development of the process control strategy for the DP is similar to what was done for the (b) (4). The QTPP was developed from the intended use, dosage strength, and container closure system, in addition to the drug product efficacy, safety and quality profile intended for the commercial product. A risk assessment was conducted to determine which potential CQAs were critical and which were non-critical, and a risk assessment was conducted to classify process parameters as either critical, non-critical or key.



A CQA was defined as any attribute that has a combined score of  $\geq 40$  based on the philosophy that attributes with high impact and high uncertainty are scored highest, attributes with high impact and low uncertainty are at intermediate values, and attributes with low impact and uncertainty are overall scored low.

*Table 28 Summary of Critical Quality Attributes and Non-Critical Quality Attributes for AVXS-101-DP*

Quality Attribute	Main QA Category	CQA/Non-CQA
Appearance (color, clarity, and visible particles)	Physicochemical	CQA
Identity by (b) (4)	Identity	CQA
Identity by (b) (4)	Identity	CQA
Identity by (b) (4)	Identity	CQA
(b) (4)	Quantity / Strength	CQA
Total Protein by (b) (4)	Quantity / Strength	CQA
(b) (4)	Quantity / Strength	CQA
(b) (4)	Quantity / Strength	CQA
(b) (4)	Potency	CQA
In vitro Relative Potency	Potency	CQA
(b) (4)	Purity	CQA
(b) (4)	Purity	CQA
% Total Purity by (b) (4)	Purity	CQA
% Total Impurities	Purity	CQA
(b) (4)	Purity	CQA
(b) (4)	Process-Related Impurity	CQA
	Process-Related Impurity	CQA
	Process-Related Impurity	CQA
	Process-Related Impurity	CQA
	Process-Related Impurity	CQA
	Process-Related Impurity	CQA
	Purity / Safety	CQA
pH	Physicochemical	CQA
Osmolality	Physicochemical	CQA
(b) (4)	Safety	CQA
Sterility	Safety	CQA
Endotoxin	Safety	CQA
Bioburden	Safety	CQA
Container Closure Integrity	Safety	CQA
Extractable Volume	Quantity / Strength	Non-CQA
In vitro assay for Viral Contaminants (b) (4),	Safety	CQA
Mycoplasma	Safety	CQA

*The classifications of quality attributes as CQA or non-CQA is appropriate.*

A process parameter risk assessment was performed via Failure Mode and Effects Analysis (FMEA), similar to what was done for the (b) (4) manufacturing. The purpose of the FMEA was to evaluate all AVXS-101 DP manufacturing process parameters with regard to risk of process failure.

The results of the FMEA concluded that the parameters in the AVXS-101 DP process were all low/medium risk, with the Overall Risk Rankings of  $\leq 21$ . The FMEA concluded that there are twelve critical process parameter (CPP) and three key process parameter (KPP) in the AVXS- 101 DP process. All critical and key process parameters are listed in Table 29.

*Table 29 AVXS-101 Drug Product Critical and Key Process Parameters*

Process Step	Critical Process Parameter	Target / Set Point	Operating Range
AVXS-101 Drug Substance	(b) (4)	(b) (4)	(4)
(b) (4)			
(b) (4)			
(b) (4)			
(b) (4)			
Filling	Fill Weight	(b) (4) (5.5 mL Label Volume) (b) (4) (8.3 mL Label Volume)	(b) (4) (5.5 mL Label Volume) (b) (4) (8.3 mL Label Volume)
Filling	Processing Time	N/A	(b) (4)
Process Step	Key Process Parameter	Target / Set Point	Operating Range
(b) (4)	(b) (4)	(b) (4)	(b) (4)

The CPP and KPP operating ranges were established from product characterization studies, development studies, validation studies, and the performance of at scale manufacturing batches.

*The classifications of process parameters as CPP or NCPP is appropriate and is summarized in Table 29 AVXS-101 Drug Product Critical and Key Process Parameters.*

### 3.2.P.2.4 Container Closure System (reviewed by AW)

Table 30 Drug Product Primary Packaging

Component	Manufacturer	Description	Process Use
Vial	(b) (4)	5 mL (b) (4) vial with a 20 mm finish	Commercial-scale DP manufacturing process for clinical supplies
	(b) (4)		
	(b) (4)	10 mL (b) (4) vial with a 20 mm finish	Commercial-scale DP manufacturing process for clinical and commercial supplies
	(b) (4)		
Stopper	(b) (4)	20 mm gray (b) (4) chlorobutyl (b) (4)	Commercial-scale DP manufacturing process for clinical and commercial supplies
Aluminum Seal with Plastic Button Cap	(b) (4)	20 mm aluminum seals with a light green button and clear lacquer in a ported bag	Commercial-scale DP manufacturing process for clinical and commercial supplies

#### Vial Selection

The selection of (b) (4) vials for the commercial-scale drug product manufacturing process for clinical and commercial supplies was based on data demonstrating durability, suitability and compatibility. (b) (4)

(b) (4) published research demonstrating the suitability of storing and shipping cell therapy products in (b) (4) vials at (b) (4)

The test concluded the (b) (4) vials were durable and break resistant after storing frozen at (b) (4) as determined by the (b) (4) vials were highly suitable as (b) (4)

(b) (4) and maintained cell viability and functionality, and the (b) (4) depending on the vial size. The (b) (4) vials also demonstrated to be optically clear, an improved (b) (4) as compared to polypropylene.

#### Quality

The integrity of the container and closure system as it relates to the prevention of microbial contamination was successfully demonstrated by the performing (b) (4) tests.

(b) (4) performed a risk-based evaluation of the extractable organic compounds detected in the extractables study. There were no observed extractable organic compounds classified as confirmed or confident for the vials. This testing supports the use of the (b) (4) vial and stopper from an extractables and leachables risk perspective.

*Reviewer comment: No significant issues with the container closure systems.*

## SECONDARY PACKAGING

Filled vials of AVXS-101 DP will be labeled and subsequently packaged with a packaging insert and alcohol wipes into cardboard cartons for 2 to 9 vials. The cardboard cartons are a two-piece full telescoping rigid set box. There are vial inserts that hold 2 to 9 vials.

### 3.2.P.2.5 Microbiological Attributes

(Reviewed by AB) The drug product is sterile filtered and aseptically filled. Drug product is tested for sterility at the time of release. Endotoxin exposure from drug product will not exceed (b) (4). The formulation does not contain a preservative.

Please refer to the DMPQ review for further information on container closure integrity testing. Container closure integrity has been demonstrated by (b) (4) tests. For stability testing, vial integrity is evaluated by (b) (4) in lieu of sterility testing.

### 3.2.P.2.6 Compatibility

(Reviewed by AB) The applicant performed two main studies to evaluate the compatibility of DP with syringes and infusion sets. These studies support instructions in the package insert to use the product within 8 h of drawing into a syringe, and to discard if not used within 8 h.

In the first study (RPT-253 in amendment 9), DP was (b) (4) (2-8°C (b) (4)) in polypropylene syringes for 8 h, followed by slow ejection through a PVC infusion set, a PVC extension set, and a winged catheter. Ejection was for up to (b) (4), followed by saline flush. The (b) (4) was measured before and after holding in syringes and ejection. The total (b) (4) in the ejection + flush was (b) (4) of the starting (b) (4). Vector activity or potency was not measured during this study.

In the second study (RPT-597 in amendment 9), DP was (b) (4) in PE syringes and passed through two types of infusion sets (PVC or PE/PVC). For the PVC infusion set, both (b) (4) potency (b) (4) were (b) (4) of the starting concentration. For the PE/PVC infusion set, only (b) (4) was measured, and the (b) (4) was (b) (4) of the starting concentration.

There were some differences between the DP used in these studies and the intended commercial DP. DP in these studies was at a slightly higher concentration (b) (4) vg/mL than commercial DP ( $2.0 \times 10^{13}$  vg/mL), and (b) (4) was at a significantly lower concentration (b) (4) than in commercial DP (b) (4).

*Reviewer comments: The data are sufficient to demonstrate compatibility of DP with administration devices for the times and temperatures that they will be used clinically. The fact that the (b) (4) in this study was lower than in the commercial product has no impact, because conducting the studies at (b) (4) represents a worst case scenario for vector adsorption. There were no compatibility studies with polycarbonate syringes, but currently-available polycarbonate syringes are small volume and very unlikely to be used to administer this product.*

#### Overall Reviewer's Assessment of Section 3.2.P.2:

The process control strategy for the DP included:

- a process parameter risk assessment via FMEA

- a risk based approach to evaluate potential CQAs to determine if they were either true CQA or non-CQAs,
- A vial withdrawal study to determine if the fill volume was justified
- Evaluation of the container closure system to evaluate the appropriateness of the vial selection , and
- Integrity testing of the container closure system to evaluate the ability of the container closure system to prevent microbial contamination.

The process control strategy for the DP is acceptable.

Components of the DP include the (b) (4), 20mM tris (pH 8.0), 1 mM magnesium chloride (MgCl<sub>2</sub>), 200 mM sodium chloride (NaCl) and 0.005% poloxamer 188. The components of the DP are appropriate quality, adequately tested and acceptable.

The applicant's primary goal for the formulation was to (b) (4). Therefore, they did not perform any formulation development except for (b) (4), with the goal of improving consistency of (b) (4) among lots and decreasing the likelihood of (b) (4).

DP manufactured under the license will include a (b) (4) to help ensure that the DP concentration remains within an acceptable range during the full 12 month shelf life.

There were early difficulties with the applicant's manufacturing process, including during the PPQ manufacturing runs. These difficulties included poor control of (b) (4), poor control of (b) (4), and poor control of DP concentration. With additional manufacturing experience, these difficulties have been resolved and the manufacturing process is currently in an acceptable state of control.

One of the central goals during the development of the AveXis manufacturing process was to produce DP that is comparable to the (b) (4). Although it is difficult to perform comparability studies when one of the manufacturing procedures is represented by (b) (4), FDA concludes that the applicant's manufacturing process produces DP with CQAs that are comparable to the CQAs of (b) (4). Extensive FDA re-analysis of the applicant's mouse survival data indicates that the applicant's lots support survival of SMNΔ7 mice to a similar extent as (b) (4), when equal amounts of vector genomes are administered to mice.

Compatibility studies were adequate to demonstrate the stability of DP when held in syringes for up to 8 h at room temperature, as well as compatibility with infusion sets.

### 3.2.P.3 Manufacture (reviewed by AW)

#### 3.2.P.3.1 Manufacturer(s)

Table 31 Drug Product manufacturers

Facility	Responsibility
AveXis, Inc. (b) (4)	Raw Material storage Excipient storage Drug Product Manufacture In-process testing Release testing Stability testing Stability sample storage Primary Packaging Secondary labeling and packaging Final QC release Finished Drug Product storage Reference standard storage
AveXis, Inc. (b) (4)	Raw Material storage Excipient storage Reference standard storage
AveXis, Inc. (b) (4)	Drug Product Release testing Stability testing
(b) (4)	Drug Product Release testing Stability testing
(b) (4)	Drug Product Release testing Stability testing
(b) (4)	Drug Product
(b) (4)	Drug Product Release testing Stability testing
(b) (4)	Drug Product Release testing
(b) (4)	Raw Material storage Finished Drug Product storage Reference Standard storage

#### 3.2.P.3.2 Batch Formula (reviewed by AW)

The AVXS-101 Drug Product (DP) manufacturing process is a batch size of up to (b) (4). The quantity of the input AVXS-101 (b) (4) lot is variable based on the yield of the AVXS-101 (b) (4).

manufacturing process. As such, the amount of drug product (b) (4) is adjusted accordingly to achieve a target concentration of  $2.0 \times 10^{13}$  vg/mL. AVXS-101 DP is filled into 10 mL (b) (4) vials with a nominal fill volume of either 5.5 mL or 8.3 mL. The commercial batch formula and quantities of each component based on fill volume are provided in Table 32 Commercial Batch Formula

Component	Quality Standard	Quantity per mL	Quantity per 5.5 mL vial	Quantity per 8.3 mL vial
AVXS-101 (b) (4)	In-House Standard	2.0 x 10 <sup>13</sup> vg	1.1 x 10 <sup>14</sup> vg	(b) (4)
Tromethamine	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Magnesium Chloride				
Sodium Chloride				
Poloxamer 188				
(b) (4)				
(b) (4)				

### Overall Reviewer's Assessment of Sections 3.2.P.3.1 and 3.2.P.3.2:

### 3.2.P.3.3 Description of Manufacturing Process (reviewed by AW)

(b) (4)

*DMPQ has resolved the issues with (b) (4).*

**(b) (4) Filling**

Once the dilution is complete, the product is filled using an (b) (4) into ready-to-use, 10 mL (b) (4), cyclic olefin polymer, vials under aseptic conditions. The (b) (4) has additional stations for stopper placement and vial capping. The vials are stoppered with a pre-sterilized ready-to-use 20 mm chlorobutyl rubber serum stopper with (b) (4). The vials are sealed with a pre-sterilized, ready-to-use packaged, aluminum seal with a colored plastic flip-off cap.

The filling machine provides an (b) (4) for the open operations which are located in a (b) (4). The filling machine is surrounded by an (b) (4). The cyclic olefin polymer vials are received pre-sterilized and double wrapped for transfer into the (b) (4). Stoppers and caps are provided in double wrapped pouches for transfer into the (b) (4).

AVXS-101 DP is filled into 10 mL vials with variable fill volumes based on the target weight. A 1.0 kg dose corresponds to a 5.5 mL nominal fill volume, and a 1.5 kg dose corresponds to an 8.3 mL nominal fill volume. The filling operation is controlled by automated recipes for the target fill weight that control the filling volumes, stopper seating and seal crimping operations. The filling machine has stations for conducting 100% in-line weight check of the filled vials as well as sensors to confirm stopper and seal placement.

**(b) (4) – Visual Inspection**

After filling, the vials are then transferred to the visual inspection area. The vials are 100% visually inspected in a (b) (4). The inspection is a manual process conducted by trained and qualified operators. Each vial is inspected for



defects, including compromised seals, incomplete closure, cracked vials, missing or incorrect container closure components, particles in solution, and foreign materials in the vial.

Following the visual inspection process the batch is sampled for AQL inspection. Visual inspected units are tested for container closure integrity utilizing a (b) (4) method. The visually inspected vials of AVXS 101 DP are forward processed to labeling or stored at  $\leq -60^{\circ}\text{C}$ .

#### **(b) (4) Labeling**

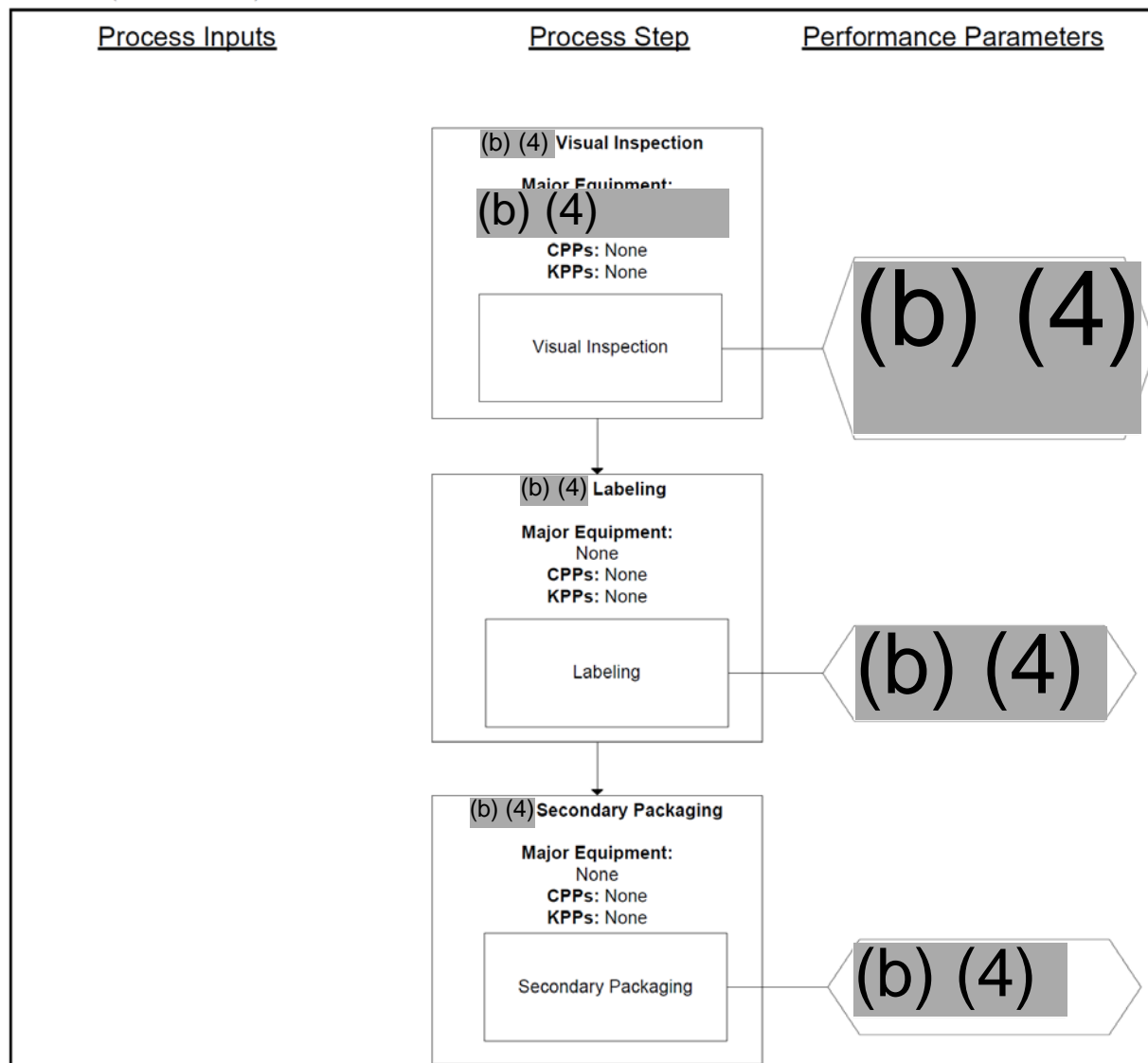
The vials are (b) (4) in accordance with master batch record instructions. Prior to the start of the batch, the label contents are inspected against approved label proofs and allocated to the batch. For vials stored at  $\leq -60^{\circ}\text{C}$  prior to labeling, the frozen state of the product is maintained through the labeling process. The vials are labeled while maintained in a (b) (4) environment during the transport and processing and removed from the (b) (4) only for wiping condensation from the vial immediately prior to label application. An AQL Inspection is conducted on labeled vials. The labeled vials of AVXS-101 DP are stored at  $\leq -60^{\circ}\text{C}$ .

#### **(b) (4) – Secondary Packaging**

Following disposition of the labeled AVXS-101 DP vials, the appropriate number of AVXS-101 DP vials are packaged in a labeled carton while maintained in a frozen state in a (b) (4) environment during processing. The commercial product kits, or Stock Keeping Units (SKU), will consist of a configuration of 1.0 kg and 1.5 kg dose volumes of AVXS-101 DP to allow for the appropriate dosing by weight of the patient. The packaged product of AVXS-101 DP is placed at  $\leq -60^{\circ}\text{C}$  until ready for distribution.

1 page determined to be not releasable: (b)(4)

Figure 39 Manufacturing flow chart part 2



**Overall Reviewer's Assessment of Section 3.2.P.3.3:**

The manufacturing process of the DP is acceptable. The DP manufacturing included a description of (b) (4) for various reasons. We discussed with the applicant during the prelicensure inspection, and the firm agreed to limited (b) (4) only for specific reasons which are now clearly defined.

**3.2.P.3.4 Controls of Critical Steps and Intermediates (reviewed by AW)**

All unit operations in the AVXS-101 DP manufacturing process are considered to be critical because it either directly impacts the AVXS-101 DP control strategy that ensures the product's critical quality attributes are achieved, or it has critical, key or performance parameters that must be achieved to ensure AVXS-101 DP conforms to defined quality attributes. The DP manufacturing process does not involve the production of any intermediates.

The DP control strategy is based on a planned set of controls derived from product and process understanding and includes:

- Controls on material attributes, including:
  - Excipients and components
  - Primary packaging materials
- Controls on the design of the manufacturing process
- In-process manufacturing controls:
  - Process Parameters
    - Critical Process Parameters (Inputs)
    - Key Process Parameters (Inputs)
  - Performance Parameters
    - In-Process Controls (Outputs)
    - In-Process Acceptance Criteria (Outputs)
- Controls on the Drug Product
- Continued Process Verification

Controls on materials used in the manufacture of AVXS-101 DP include control of the excipients, components and the control of the primary packaging materials.

- The quality and control of the excipients is reviewed above in section 3.2.P.4 below.
- Controls on the single use and major process equipment used in DP manufacturing were also provided. The (b) (4) equipment surfaces that contact the sterilized DP include the filtered drug product bag and filling needle assembly which are both single use components. Manufacturing equipment surfaces that contact the sterilized containers, vial closures or are near the sterile product are routinely monitored for contamination. (b) (4) parts are (b) (4) using validated (b) (4), and are tested for microbial contamination prior to each product fill.
- Controls of the primary packaging materials include:
  - a. quality control testing by each supplier,
  - b. review of the quality certificate by AveXis quality control, and
  - c. incoming confirmatory testing for sterility and endotoxin for each lot of the 10mL (b) (4) vials, 20mm Stopper, and 20 mm seal. by AveXis quality control

### **Controls on the Design of the Manufacturing Process**

The AVXS-101 DP manufacturing process control strategy, which includes determination of process parameter criticality and establishment of key process parameters and critical process parameters, is described in this section 3.2.P.2.3.

### **In-Process Manufacturing Controls**

The development of the in-process manufacturing controls is described in Module 3.2.P.2.3 of the BLA. The data confirming that the overall process control strategy is appropriate for achieving and maintaining the defined quality and yield of the AVXS- 101 DP are presented in Module 3.2.P.3.5 of the BA

A risk-based approach was adopted for the assignment of CQA's, similar to the principles outlined in the A-Mab: A Case Study in Process Development, CMC Biotech Working Group, published by ISPE, Version 2.1, Oct 2009 and A-Vax: Applying Quality by Design Principles to Vaccines, CMC-Vaccine

Working Group, by Parenteral Drug Association. The outcome of the risk-based approach is summarized in Table 28.

*The development / validation data support the selection and justification of the CPP, KPP, IPC, and ranges. The control strategy is appropriate to assure product quality and process consistency as well.*

**Overall Reviewer's Assessment of Section 3.2.P.3.4:**

The controls of the critical steps in manufacturing are acceptable. The control strategy is appropriate to assure product quality and process consistency as well.

**3.2.P.3.5 Process Validation and/or Evaluation** (reviewed by AW)

The PPQ was performed at commercial scale at AveXis in (b) (4).

The AVXS-101 DP manufacturing process was qualified from the (b) (4). In addition to qualifying the production process, an (b) (4) and an additional sterile filtration step were each included during one of the PPQ runs. These steps are part of the routine process control strategy in the event that the concentration of the (b) (4) exceeds the In-Process Control Limit for (b) (4).

*Reprocessing including an additional sterile filtration step are approved in three specific situations. All others will require a prior approval supplement.*

A prospective protocol (PRO-801) was written which defined the sampling, analytical testing plan, and acceptance criteria for each process step. The AVXS-101 DP labeling and secondary packaging operations were outside the scope of the PPQ protocol.

**Critical Process Parameter Evaluations**

(b) (4)

[REDACTED]



(b) (4)

(b) (4)

**Fill Volume Justification**

The applied target fill volumes is based on the concentration of the DP and the dose. The fill volume accuracy on the vial filler and the volume capable of being withdrawn from the vial were assessed to determine the target fill volume and allowable range to meet the volume label claim and consistently achieve the fill volume for the release of AVXS-101 DP.

The target fill volume and volume withdrawn from vials containing (b) (4) were tested to mimic filling and withdraw procedures for AVXS-101 DP. The AveXis-(b) (4) filler was used to load, fill,

stopper and cap/seal 10 mL Ready-to-Use (b) (4) vials. (b) (4) target fill weights of (b) (4) were evaluated for filler accuracy.

- (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

The commercial label claim for the AVXS-101 DP presentations are 5.5 mL and 8.3 mL. The Drug Product density of (b) (4) translates these volumetric label claims to a weight of (b) (4), respectively. Therefore, in order to consistently achieve the labeled volume with withdrawal loss and filler control considerations a target fill weight of (b) (4) was determined as provided in Table 35.

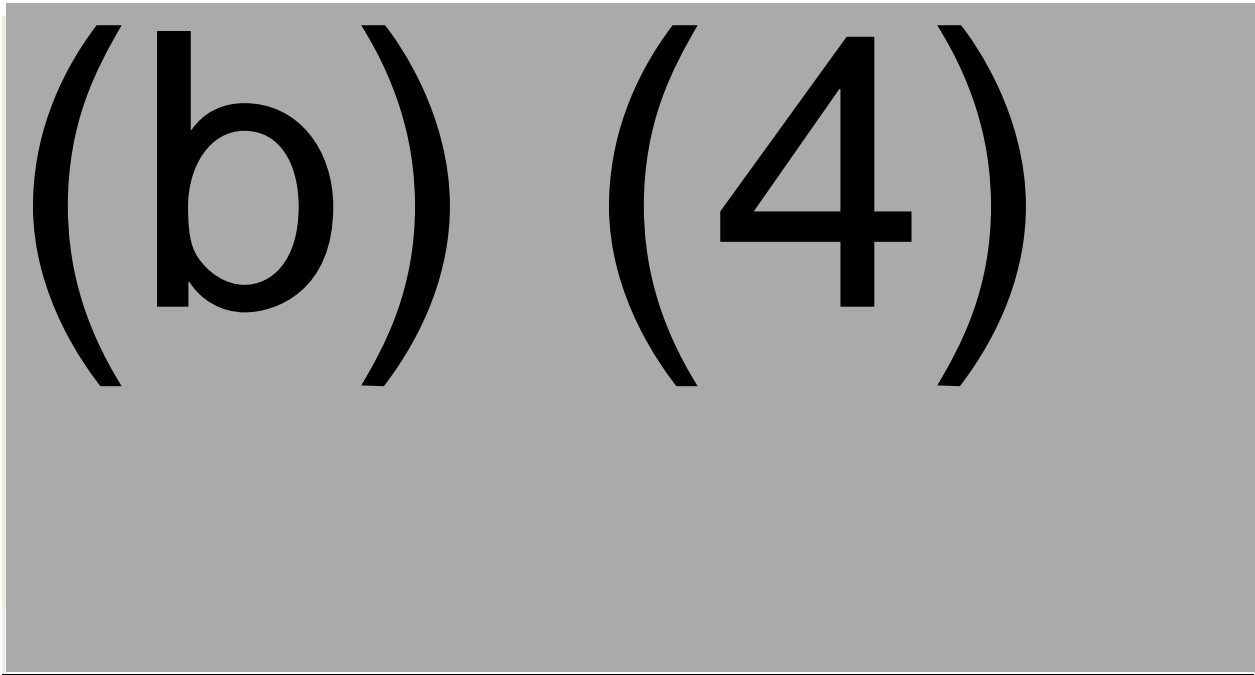
*The fill volume justifications are appropriate.*



*During the prelicensure inspection FDA inspectors observed the labeling procedures. FDA recommended the firm develop proficiency testing for operators to confirm they can correctly apply a label to a frozen vial. There is also a concern that the labels may lose adhesiveness over time while frozen. This would be best addressed by adding a label criteria to the stability protocol. This was discussed with the applicant on May 1, 2019. The applicant was sent information request 62 on May 1, 2019 and we requested the executed report demonstrating that the labeling process is under control. The information was due on May 10 and was received on May 10 in amendment 81. In this amendment the applicant provided RPT 1121 which includes a summary of the validation study of labeling of the DP and is reviewed below.*

RPT-1121 – Validation Study: Labeling of AVXS-101 Drug Product contains a summary of the validation study to evaluate the labeling of the frozen DP vials. In this study the applicant filled 10mL vials with 5.5 mL (b) (4) which was used as a surrogate for the DP and froze the vials at  $\leq -60^{\circ}\text{C}$  storage for no less than (b) (4). The frozen vials were labeled with according to the standard procedures and inspected to check for defects listed in Table 36 DP Labeling Validation Study Results. There were no defects identified in the (b) (4) vials inspected.

*Table 36 DP Labeling Validation Study Results*



*RPT- 1121 is acceptable and demonstrates that the applicant has control over the labeling procedure.*

The manufacturing process was qualified at commercial scale with a series of (b) (4) batches: (b) (4) fill volumes. The batch size for was (b) (4) for each three of the batches and one of the (b) (4) fill volumes included a batch size of (b) (4).

During each of the qualification runs the applicant was able to meet the prespecified operating ranges for each process parameters.

*Reviewer Comments: The study described did not evaluate the ability of the labels to remain attached to the vials after being frozen at  $\leq 60^{\circ}\text{C}$  long term. We discussed this concern with the applicant during a T-con on May 2, 2019. In amendment 81 received on May 15, 2019 the applicant provided protocol PRO-931 AVXS-101 Drug Product Vial Labeling and Long term Storage Validation Study and RPT-1394 AVXS-101 Drug Product Vial Labeling and Long term Storage Validation Study Interim Report.*

*PRO-931 described the plan to evaluate the ability of the labels to remain attached while under long term storage at  $\leq 60^{\circ}\text{C}$  and RPT 1394 provided results for the first 7 days. In the study, the applicant labeled (b) (4) held them at  $\leq 60^{\circ}\text{C}$  and will evaluate if the labels remain attached to the vial at various intervals over (b) (4) as listed in Table 37. The plan is reasonable and will adequately address our concerns about the ability of the labels to remain attached to the vials long term.*

Table 37 Labeling and long-term storage plan

(b) (4)
---------

*Reviewer Comments: The qualification demonstrated that the applicant was able to manufacture the DP. The data provided supports the drug product operating ranges, and parameters are adequate.*

#### **Continued process verification** (reviewed by AB)

The original BLA submission did not have a plan for continued process verification (CPV), and we listed the lack of a CPV plan as a deficiency in the filing letter. In amendment 21 (January 17, 2019), the firm provided PLAN-244 (AVXS-101 Drug product continued process verification plan). Process data from DP lots will be reviewed every (b) (4) or every (b) (4) (whichever is shorter) for trends and to evaluate the continued appropriateness of the control limits or acceptance criteria. In addition, all non-conformances will be analyzed. A report will be written and additional actions will be identified if needed.

In IR#32 (sent February 1, 2019), we requested that CPV plans be updated to incorporate a pre-defined statistical approach to detecting non-random effects. In amendment 47 (March 15, 2019), the firm revised all CPV plans to include use of Nelson control rules 1 through 4. The Nelson rules will be applied to run charts once approximately (b) (4) lots of data have been collected.

*Reviewer comment: The revised CPV plans are acceptable.*

#### **Overall Reviewer's Assessment of Section 3.2.P.3.5:**

The data provided support the drug product operating ranges, and parameters are adequate.

### 3.2.P.4 Control of Excipients (reviewed by AW)

#### 3.2.P.4.1 Specifications

All excipients used in the manufacture of AVXS-101 Drug Product (DP) are of compendial grade as presented in *Table 38 Excipient Quality and Confirmatory testing*. Representative vendor certificates of analyses are provided for each excipient as an attachment.

*Table 38 Excipient Quality and Confirmatory testing*

Excipient	Quality Standard	Additional Testing Performed
Sodium Chloride	(b) (4)	Appearance, Identity, Endotoxin
Tromethamine	(b) (4)	Appearance, Identity, Endotoxin
Magnesium Chloride	(b) (4)	Appearance, Identity, Endotoxin
(b) (4)	(b) (4)	Appearance, Identity
Poloxamer 188	(b) (4)	Appearance, Identity
(b) (4)	(b) (4)	Appearance, Conductivity, Endotoxin, Nitrates, Total Organic Carbon

*Reviewer's Comment: The excipients used are appropriate quality and are adequately tested.*

#### 3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures

Not applicable

#### 3.2.P.4.4 Justification of Specifications

Not applicable

#### 3.2.P.4.5 Excipients of Human or Animal Origin

Not applicable

#### 3.2.P.4.6 Novel Excipient

Not applicable

#### Overall Reviewer's Assessment of Section 3.2.P.4:

The Excipients used are of appropriate quality and are adequately tested.

### 3.2.P.5 Control of Drug Product

#### 3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)

(Reviewed by AB) The statistical approach to setting limits for DP is the same as described in 3.2.S.4.5 for (b) (4). However, there were many fewer DP lots (b) (4) in the original BLA submission, and therefore the tolerance interval (TI) approach yields wider intervals (relative to 3SD) for the DP than for the DS. When needed, FDA requested updated data from additional DP lots.

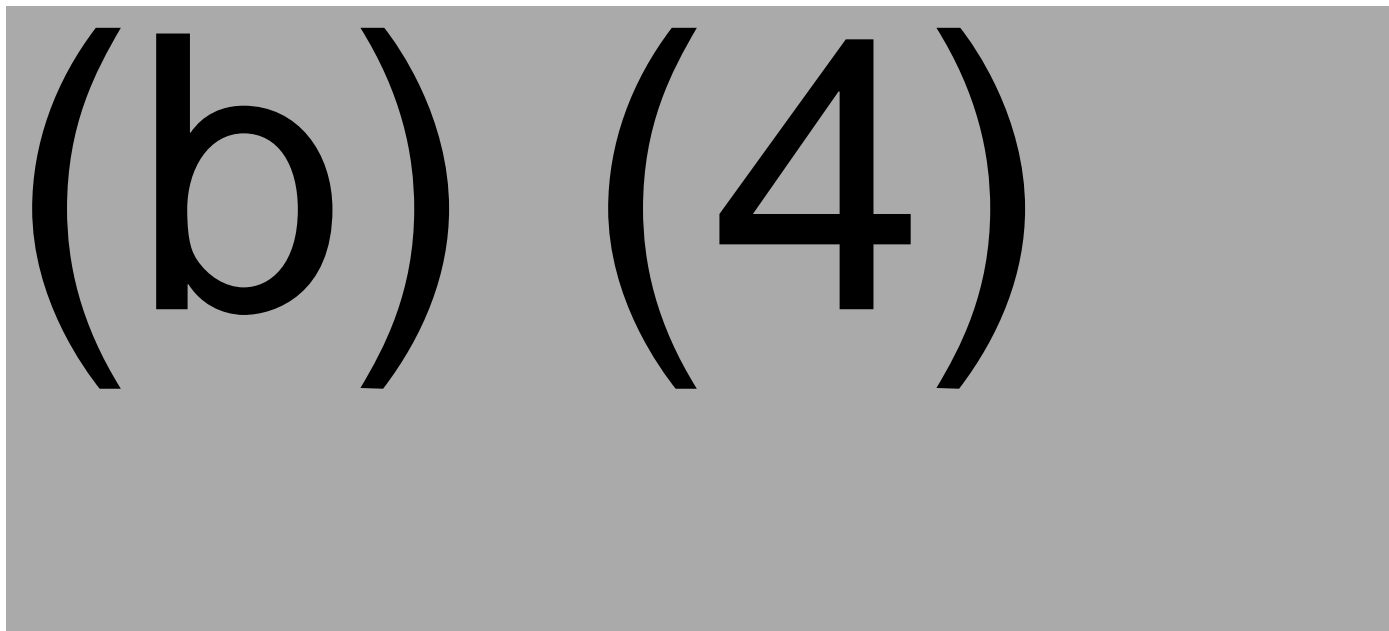
For certain quantitative assays that are performed on (b) (4) DP, the acceptance criteria are identical for (b) (4) DP (pH, osmolality, protein purity by (b) (4)).

Table 39 DP specifications

Test Parameter (Attribute)	Analytical Procedure	Final Acceptance Criteria	Justification for Specification	Clinical Lot AAV9SMN0619 Acceptance Criteria	PPQ/Validation Lots Acceptance Criteria
Appearance	(b) (4) SOP-345	Clear to slightly opaque, colorless to faint white solution, free of visible particulates	(b) (4)		
pH	(b) (4) SOP-057				
Osmolality	(b) (4) SOP-128				
(b) (4)	(b) (4) SOP-262				
(b) (4)	(b) (4) SOP-137				
(b) (4)	(b) (4) SOP-328				
Total protein	(b) (4) SOP-184				
(b) (4)	(b) (4) D SOP-259				
Potency (b) (4)	(b) (4) Δ7SMA mouse SOP-346				
Potency (b) (4)	(b) (4) SOP-347				
Identity (DNA)	(b) (4) SOP-137				
Identity (Protein)	(b) (4) SOP-180				

Identity (Protein)	(b) (4)
Purity (b) (4)	
Purity (Protein)	
Impurities (Protein)	
Endotoxin	
Sterility	

#### Additional analysis of quantitative DP specifications



*Figure 42 pH*

The pH of AAV9SMN0613 DP was (b) (4). Initial FDA analysis found that the data were not normally distributed. In response to information request #17, the applicant submitted pH data in amendment 41 from (b) (4) additional DP lots. These new data demonstrate that the pH values are normally distributed and confirm that the proposed specification of (b) (4) is appropriate. This is the same pH acceptance criterion as for (b) (4).

(b) (4)

*Figure 43 Osmolality*

The osmolality of (b) (4) DP was (b) (4). The acceptance criterion of (b) (4) is reasonable (this is the same as the acceptance criterion for osmolality of (b) (4)).

(b) (4)

(b) (4)

(b) (4)

(b) (4)



### **Overall Reviewer's Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6:**

The DP specifications provide adequate control of the quality of the strength, identity, purity and potency of the DP.

Strength is measured by (b) (4). The stability data indicate that the strength is declining at a rate of about (b) (4) per year, and a minimum strength of the commercial DP will be assured by the minimum acceptance criterion of (b) (4) vg/mL, which is just (b) (4) below the nominal concentration of  $2.0 \times 10^{13}$  vg/mL. New lots manufactured under the license will also include a (b) (4) overage (target concentration of (b) (4) vg/mL), which will help to ensure that the strength remains in an appropriate range throughout the entire 12 month shelf life.

The vector DNA identity is controlled by the (b) (4) assay, which detects a sequence that is specific to onasemnogene abeparvovec-xioi. The capsid protein identity is controlled by the (b) (4) assays. These protein assays cannot distinguish between AAV9 proteins and proteins from other AAV serotypes, but onasemnogene abeparvovec-xioi is currently manufactured in a dedicated facility, and thus there is no concern that the capsid proteins might be derived from a non-AAV9 serotype.

Purity is mainly controlled in the (b) (4), but a few tests are performed on DP. DP is tested to ensure the absence of microbial contaminants and to ensure that endotoxin does not exceed acceptable limits. Importantly, the amount of (b) (4) is controlled by the analytical (b) (4) assay, which also likely provides a degree of control over (b) (4).

Potency is controlled primarily by the (b) (4) potency assay. This assay quantifies the ability of the test article to produce SMN protein in cells, relative to the amount of SMN protein produced by a reference standard vector. The acceptance criteria are relatively narrow for a biological assay (b) (4), which provides good control, but there is some concern that the potency of the reference standard vector may not be stable during long-term storage. Potency is also controlled by an in vivo assay that evaluates the ability of DP to enhance survival in a mouse model of SMA. This is an excellent assay from a mechanistic standpoint and is the only assay that measures functionality of the SMN protein produced by the vector, but the assay has low sensitivity. Finally, the (b) (4) assay measures the infectivity of DP, but has high variability and wide acceptance criteria. Historically, the potency of AveXis lots have been consistent and comparable to lot AAV9SMN0613, including in relatively large-scale mouse survival studies that were performed in 2017 and 2018 to compare early AveXis DP lots to lot AAV9SMN0613.

The quality and concentration of the DP excipients are controlled by the pH, osmolality and (b) (4).

### **3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures**

(Reviewed by AB) The following five assays are performed for release of (b) (4) DP, and are reviewed under 3.2.S.4.2 and 3.2.S.4.3:

- Appearance (SOP-345)
- pH (SOP-057)
- Osmolality (SOP-128)



- (b) (4) (SOP-137)
- Purity and impurity by (b) (4) (SOP-180)

Acceptance criteria for these five assays are the same for (b) (4) DP, with the exception of appearance: the acceptance criterion for DP requires that there be no visible particulates (DP is 100% visually inspected), but there is no such criterion for DS.

(b) (4)

(b) (4)



(b) (4)

**Endotoxin per (b) (4) (SOP-121 v5.0)**  
Reviewed by DBSQC.

**Sterility per (b) (4) (SOP 337 v1.0)**  
Reviewed by DBSQC.

**Container closure integrity per (b) (4) (SOP-312 v3.0)**  
Reviewed by DMPQ.

**Overall Reviewer's Assessment of Sections 3.2.P.5.2 and 3.2.P.5.3:**

Assays for release of DP have been appropriately designed and validated. The only exception is the (b) (4) assay, which still needs to be validated for robustness and requires a system suitability criterion to set limits on the titer of the assay reference standard. Resolving these issues with the (b) (4) assay will be PMC #3.

During review of the BLA, the analytical (b) (4) assay underwent substantial changes to improve the assay, and these changes required recalculating the acceptance criteria

for this assay. Also during review of the BLA, the total protein assay was revalidated because the initial validation was not performed correctly and gave an incorrect estimate of the precision. The revalidation demonstrated that the total protein assay is less precise than initially thought.

#### 3.2.P.5.4 Batch Analyses

(Reviewed by AB) This section contains a history of the DP specifications. Major changes to DP specifications include:

- (b) (4)

The COAs for the PPQ lots are included in this section (DP lots (b) (4)). There is also a summary table including all lot release data from (b) (4). Data from all lots are discussed more comprehensively in 3.2.S.4.5 and 3.2.P.5.6.

(b) (4) was performed on two of the PPQ lots. (b) (4) lot (b) (4) was too (b) (4) and was (b) (4). PPQ lot (b) (4) was found to be (b) (4). The applicant agreed during the (b) (4) inspection and (in amendment 60) not to perform this (b) (4) for the purposes of (b) (4) in the future. (b) (4) will be allowed under certain specific circumstances (for example, a lead during (b) (4) that might compromise sterility).

Information submitted in RPT-1320 in amendment 60 demonstrates that there is no deleterious impact on the DP assay values for (b) (4) DP lots that were (b) (4) these lots were (b) (4) (PPQ), (b) (4).

#### 3.2.P.5.5 Characterization of Impurities

(Reviewed by AB) No new process-related impurities are introduced during DP manufacture.

Several product-related impurities are evaluated in DP. Capsid forms are evaluated using the (b) (4) assay, and protein impurities are evaluated using the (b) (4) assay. (b) (4) DP are evaluated using the (b) (4) assay, and are subject to the same lot release criteria. There is no indication of any change in protein purity (b) (4) DP.

#### Overall Reviewer's Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5:

Process-related impurities are controlled in the (b) (4), and no new impurities are introduced during manufacturing of DP. There were a number of major changes to DP release assays, especially the analytical (b) (4) assay, which quantitates the various (b) (4) full capsids forms in this product. The new (b) (4) assay was introduced very late in product development, and the in vivo potency assay was revised to a less complicated assay that requires fewer mice.

(b) (4) of DP will be allowed under certain very limited circumstances, and the applicant has submitted acceptable evidence that (b) (4) does not have a negative impact on DP.

Overall, control of DP is adequate, and the product has consistent strength, potency and purity.

### 3.2.P.6 Reference Standards or Materials (reviewed by AB)

(b) (4)

*Reviewer comment: In amendment 80 (March 13, 2019), the applicant explained that there is no specific stability protocol for the current reference standard RS-002 because RS-002 is derived from lot (b) (4), and lot (b) (4) is already on the stability program. In the event that a future reference standard is not already on stability, a stability protocol will be established. This is acceptable.*

Qualifying a new primary reference standard or working reference standard must be performed according to an approved protocol. Protocol PRO-804 and a number of other documents related to the reference standard were extensively reviewed during the AveXis (b) (4) inspection. If the in vitro potency for the new reference standard is found to be within acceptable limits relative to the old reference standard (using a (b) (4) equivalency statistical approach), the new reference standard will be assigned a potency of 100%.

### 3.2.P.7 Container Closure System (reviewed by AW)

Table 40 Container Closure System Components

Component	Manufacturer Product Number	Manufacturer Name and Address	Description	DMF/Letter of Authorization
Vial	(b) (4)			
Stopper				
Aluminum Seal with Plastic Button Cap				

#### Component Specifications

##### Vial

168 vials are packaged in a high density (b) (4) ) tray/ lid inside a sealed (b) (4) with a secondary (b) (4) . The vials and tray/ lids are (b) (4) Sterilized. (b) (4) are placed in a poly-lined carton. A diagram and dimension attributes for the DP vial are provided for the Vial.

Table 41 Vial Release Specifications

Test	Method	Acceptance Criteria
Identification/Chemical	(b) (4)	(4)
Visual Defects		
Sterilization Dose Range (kGy)		
Endotoxin(EU/mL)		

#### Stopper

The (b) (4) 20 mm stopper is compression molded from (b) (4) chlorobutyl rubber. Formulation characteristics are describe in COA (b) (4) from (b) (4) for the Specification for Stopper Formulation (b) (4) Gray and the cross referenced master files for stopper and stopper formulation in the table above showing stopper release specifications. A diagram and dimensional attributes for the stopper are provided. (b) (4) for Stopper – 20mm (b) (4). The stoppers are (b) (4)

The materials of construction for vials and stoppers comply with the current version of the (b) (4). AveXis will use the ANSI/ASQ Z1.4, Special Inspection Level S-3 sampling plan for dimensional analysis and ANSI/ASQ Z1.4, General Inspection Level II Single Normal Plan for Visual Inspection. Quality Assurance will determine the sample size by referring to QAD for quantity of lot/batch. The number of components sampled and inspected will be pulled from a minimum of (b) (4). The specifications for the vials are provided in the next table. A sample Quality Certificate with test results is presented in a COA.

#### Seal

The primary container closure is sealed with an aluminum 20 mm flip-off seal with a colored plastic button cap produced by (b) (4). The (b) (4) seals consist of an aluminum shell and a plastic (polypropylene) button that are tamper-evident. The seals are manufactured with (b) (4), which assures that the seals meet tight dimensional standards. The seals are cleaned, sterilized, certified and provided in a ready to use format.

The applicant conducts confirmatory testing for sterility and endotoxin is conducted on each component of the container closure system (vial, stopper and sealer).

*No deficiencies in the container closure system.*

#### **Overall Reviewer's Assessment of Section 3.2.P.7:**

The container closure system includes a 10mL (b) (4) vial, a 20mm stopper and an aluminum seal. All (b) (4) are pharmaceutical grade, and have been tested and shown to be appropriate.

### 3.2.P.8 Stability

#### 3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data (reviewed by AB)

The original BLA submission contained just (b) (4) months of stability data with AveXis DP lots at the long-term  $\leq -60^{\circ}\text{C}$  storage condition, which was insufficient to analyze. FDA filed the application even with this major deficiency, with the expectation that additional stability data would be provided during the BLA review period. On March 29, 2019, the applicant submitted amendment 53 (RPT-411), which contains up to 1 year of stability data from (b) (4) AveXis DP lots, and up to (b) (4) of stability data from lot AAV9SMN0613 covering the period when this lot was (b) (4) months old.

Lot AAV9SMN0613 was manufactured in December, 2013 and used for the phase I study CL-101. AAV9SMN0613 was evaluated for stability at NCH several times through September, 2016 (b) (4) months after manufacturing). These data were previously submitted to IND 15699 and indicated losses of approximately (b) (4) for both vector genome concentration and (b) (4). However, further investigation revealed that the assays performed by NCH were unreliable. FDA allowed phase 3 clinical trials with AveXis lots to proceed only after AveXis provided an additional (b) (4) of stability data with lot AAV9SMN0613 (February, May and August, 2017). These new AveXis stability data indicated that (b) (4) of AAV9SMN013 were stable for (b) (4), although the conclusion was not robust due the limited number of data points, the limited length of the study, and the fact that only one lot was analyzed for stability.

RPT-411 contains (b) (4) of stability data for lot AAV9SMN0613, acquired using AveXis assays between February, 2017 and May, 2018. One limitation is that the in vitro potency assay was developed fairly late, and there was only a single value obtained for AAV9SMN0613 (73%, in May of 2018). Another limitation is that the (b) (4) assay was changed in August, 2017 to produce more accurate results. Thus, the data for AAV9SMN0613 vg concentration only span August, 2017 to May, 2018 (9 months). Based on discussion during the AveXis (b) (4) inspection, in (b) (4) the applicant added another data point for AAV9SMN0613 vg concentration and potency, sampled from an original container instead of an aliquot (amendment 60, April 11, 2019). This March, 2019 value (b) (4) vg/mL) is not shown in the analyses below, but is consistent with a decline in vector genome concentration for lot AAV9SMN0613 (compare to (b) (4) vg/mL in August, 2017).

Samples for stability were aliquoted into small-volume (b) (4) vials, which have the same material composition as the final container (10 mL (b) (4) vials). However, the ratio of surface contact area to product volume is higher, and the headspace volume is smaller.

AveXis lots in the RPT-411 stability study included (b) (4) PPQ lots that were at a concentration of about (b) (4) vg/mL, with 12 months of data currently available (Figure 57). At the commercial concentration of  $2 \times 10^{13}$  vg/mL, there are stability data for all (b) (4) PPQ lots (12 months) and (b) (4) post-PPQ lots (6 months). All lots were evaluated for stability at  $\leq -60^{\circ}\text{C}$ ,  $2-8^{\circ}\text{C}$  and (b) (4), except for AAV9SMN0613, which was evaluated at only  $\leq -60^{\circ}\text{C}$  (Figure 58). The stability test limits were the same as the proposed DP lot release specifications (Figure 59). Note that not all of the assays were performed at each time point.



Process	DP Lot Number	DS Lot Number(s)	Final Vector Concentration (vg/mL)	Container Closure System	Fill Date	Fill Volume	Units Filled	Lot Use	
Process A	(b) (4)	(b) (4)	1.1 x 10 <sup>13</sup>	2 mL and 5 mL Polypropylene Round Bottom Tube	(b) (4)	(b) (4)	(b) (4)	Phase 1 Clinical	
Process B – Pre-PPQ			(b) (4)	(b) (4)				5 mL (b) (4) Cyclic Olefin Polymer Vial; 20 mm Stopper; 20 mm Seal	Clinical Trial, Stability
								Clinical Trial, Stability	
								Clinical Trial, Non-clinical, Stability	
Process B – PPQ								10 mL (b) (4) Cyclic Olefin Polymer Vial; 20 mm Stopper; 20 mm Seal	Clinical Trial, Stability, Process Validation
									Clinical Trial, Stability, Process Validation
									Clinical Trial, Stability, Process Validation
									Clinical Trial, Stability, Process Validation
Process B – Post-PPQ								10 mL (b) (4) Cyclic Olefin Polymer Vial; 20 mm Stopper; 20 mm Seal	Commercial, Stability
	Commercial, Stability								

Figure 57 DP lots in stability report RPT-411

Study Protocol Number	Item Number	Drug Product Lot	Study Length		
			Long Term ≤ -60°C	Accelerated 2-8°C	(b) (4)
<sup>2</sup> PRO-218	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
PRO-410					
PRO-436					
PRO-451					
<sup>1</sup> PRO-462					
<sup>1</sup> PRO-498					
<sup>1</sup> PRO-498					
<sup>1</sup> PRO-498					
PRO-578					
PRO-578					

<sup>1</sup>AVXS-101 DP lots included in this protocol were manufactured per PLAN-171 Filling/Finishing Process Performance Qualification (PPQ) Plan for GMP Commercial Production of AVXS-101.

<sup>2</sup>Manufacture date for lot AAV9SMN0613 was (b) (4). This stability study began on 08Feb2017. Per PRO-218, T=0 will be the results using the then newly developed analytical methods.

Figure 58 DP stability study protocols

Test Name	Location	Test Method Reference	Test Limits
(b) (4)	(b) (4)	SOP-137	(b) (4)
Appearance by Visual Inspection		<sup>1</sup> SOP-345/ SOP-164	
pH		SOP-057	
Osmolality by (b) (4)		SOP-128	
<sup>40</sup> % Total Purity by (b) (4)		SOP-180	
<sup>4</sup> Total Impurities by (b) (4)		SOP-180	
(b) (4)		SOP-192/ <sup>2</sup> SOP-328	
In-vitro Potency <sup>3</sup>		SOP-347	
CCIT - (b) (4)		SOP-312	

<sup>1</sup>See CCR-161 for the change in appearance method from SOP-164 to SOP-345.

<sup>2</sup>Testing site was transitioned to (b) (4) for new studies in June 2018.

<sup>3</sup>As of May 2018, (b) (4) potency replaced In vivo Potency by Δ7SMN mice per CCR-246.

<sup>4</sup>Specification updated on 29Oct2018 to include increased precision with an additional decimal place.

<sup>5</sup>SPEC-123 v10.0 previously included P/N: 4111/4112 and retained the same (b) (4) specifications as P/N: (b) (4)

Figure 59 DP stability specifications

#### DP stability at the long-term $\leq -60^{\circ}\text{C}$ storage condition

Vector genome concentration declines over time for all (b) (4) DP lots, and the rate of decline appears to be similar for all lots (Figure 60). None of the lots formulated at the  $2.0 \times 10^{13}$  vg/mL concentration fell below the 1 (b) (4) vg/mL lower limit, but there were multiple data points on the lower specification line. It is worth noting that the vg concentrations are reported with only 2 significant digits, so values in the range of  $1.65\text{--}1.69 \times 10^{13}$  vg/mL will be rounded up to (b) (4) vg/mL and will pass.

1 page determined to be not releasable: (b)(4)

(b) (4)

FDA discussed DP stability with the applicant in teleconferences on April 9, 2019 and April 16, 2019. During the April 16 teleconference, the applicant provided an alternate model for the stability of vg concentration (“stability model B”) where the independent variable is the square root of time. This model predicts a rapid decrease in vg concentration during the first year, with a much slower rate of decay in additional years. FDA informed the applicant that although the alternate model might be a good mathematical fit, the alternate model was not adequately supported by data, and it is difficult to understand the biological basis for the rate of decline varying in a manner that depends on the square root of time.

In IR #54 on April 25, 2019, FDA asked the applicant to raise the DP lower release limit for vg concentration to (b) (4) vg/mL, in order to ensure that the DP strength remains within an appropriate range throughout the full 12 month shelf life. The applicant agreed in amendment 73 (May 6, 2019). During a teleconference on May 2, 2019, the applicant noted that they have several DP lots in storage (originally intended for launch) that are very close to the 12 month expiration date. They asked to re-test these lots for vg concentration and in vitro potency to ensure that they were still within specification, and if so these lots would then receive a 12 month shelf life from the date of the re-test. If a re-tested lot was found to have a vg concentration between (b) (4) vg/mL, it would receive a shortened shelf life, with the number of months based on prediction by the applicant’s stability model B (from the April 16, 2019 telecon). These re-tests and shelf life extensions would only be carried out on lots prior to licensure; after licensure, all lots would receive a shelf life of 12 months. On May 7, 2019 (IR #62), FDA informed the applicant that this plan is not acceptable. The shelf life will be 12 months from the date of fill for all DP lots, and re-testing will not be allowed.

*Potency (b) (4) at the long-term storage temperature*

The in vitro potency also declines during long-term storage at  $\leq -60^{\circ}\text{C}$  (Figure 63). The in vitro potency assay measures the ability of the vector to express immunoreactive SMN protein, as compared to reference standard vector RS-002 that has an assigned value of 100% for potency. The in vitro potency assay was implemented only in May, 2018 (after the stability study had started), so the data are incomplete. None of the AveXis lots in this study fell below (b) (4) potency.

One serious difficulty in interpreting the potency data is that the reference vector RS-002 is likely declining in concentration and potency with time. RS-002 was created by aliquoting lot (b) (4) in early 2018. When lot (b) (4) was manufactured in December, 2017, it had a concentration of (b) (4) vg/mL.

1 page determined to be not releasable: (b)(4)

During the AveXis (b) (4) inspection and at the late cycle meeting, FDA requested additional potency and vg concentration data from lot AAV9SMN0613. In March, 2019 (RPT-1333, amendment 60, April 11, 2019), an (b) (4) of AAV9SMN0613 was assayed for vg concentration and in vitro potency. The (b) (4) concentration was (b) (4) vg/mL, indicating a decline in concentration of (b) (4) from the (b) (4) vg/mL value measured in August, 2017. Using this (b) (4) to calculate the appropriate (b) (4) to use in the in vitro potency assay, the potency was measured (b) (4) and returned a mean of (b) (4) potency.

During the same assay (RPT-1333), the concentration and potency of lot (b) (4) were measured from an (b) (4) DP vial. The concentration of (b) (4) measured in March, 2019 was (b) (4) vg/mL, indicating a decline of (b) (4) as compared to the original concentration of (b) (4) vg/mL that had been measured in June, 2018. The in vitro potency of (b) (4) had been measured once before: in December, 2018, the potency was (b) (4). In March, 2019 the potency was measured (b) (4), using the vg concentration of (b) (4) vg/mL) and returned a mean potency of (b) (4).

*Reviewer comment: The concentration and potency measurements in March, 2019 (RPT-1333) confirm ongoing decline in vector concentration in lots AAV9SMN0613 (b) (4) decline over (b) (4) months) and (b) (4) decline over (b) (4) months), even when the samples are acquired from (b) (4) DP containers. This result suggests that the decline in concentration cannot be attributed to the (b) (4) process that was used to prepare other samples for stability testing.*

*The potency results in RPT-1333 suggest that the potency per unit vg remains relatively constant. In other words, when the correct (b) (4) is used in the potency assay, the potency is close to (b) (4). Although the in vitro potency assay is variable and it is difficult to draw firm conclusions about changes in potency, this finding suggests that the potency is not declining at a faster rate than the vg concentration is declining.*

The (b) (4) is also declining over time in all (b) (4) DP lots on the stability study (Figure 65). No lots fell below the lower acceptance limit, but the specification is very wide. When a global fit is performed (Figure 66), the decay rate is (b) (4).

This (b) (4) rate should be viewed with a great deal of caution due to the low  $r^2$  value and high variability of the assay. This type of assay is also susceptible to (b) (4), and the (b) (4) assay has not been controlled for (b) (4) in the past. In IR #20 on 12/28/18, we requested that the applicant add a system suitability criterion to this assay to ensure that the reference control vector would fall within a certain range. This issue with control of the (b) (4) assay was not addressed during the BLA review and will be PMC #3.

(b) (4)

*Other assays*

In addition to the three assays above, DP lots on the stability protocol were evaluated for appearance, pH, osmolality, and (b) (4) purity. No adverse trends were identified. The purity as measured by (b) (4) remains constant, indicating that capsid proteins are not being degraded at the long-term storage temperature. Table 42 shows (b) (4) stability of lot AAV9SMN0613, and other lots had similar profiles. The total impurities and the individual impurities fluctuate from assay to assay, but there is no overall trend.

(b) (4)

AAV9SMN0613 was also evaluated for in vivo potency at multiple time points (Figure 37) – no adverse trend was identified for median survival in groups of SMNΔ7 mice, but comparing median survival is a very insensitive way to detect changes in potency.

*Reviewer comment: The stability data indicate that DP is losing vector genome concentration, potency and (b) (4) when stored at  $\leq -60^{\circ}\text{C}$ . The best estimate of the rate is (b) (4) loss per year (95% CI = (b) (4) per year), and this rate was obtained from the vector genome concentration for (b) (4) lots, with data for up to 1 year.*

*Data from the in vitro potency (b) (4) assays suggest a possible risk that the rate of decay in vector potency might be greater than (b) (4) per year. However, data from the in vivo potency assays suggest that the ratio of potency to vector genomes remains stable over time. During 2017 and early 2018, newly-manufactured AveXis lots were compared head-to-head in SMNΔ7 mice with lot AAV9SMN0613 (based on the August, 2017 AAV9SMN0613 concentration of (b) (4) vg/mL). In spite of the fact that lot AAV9SMN0613 was about (b) (4) years old at the time of the in vivo assays, the ability of AAV9SMN0613 to rescue survival of mice followed the same dose-response relationship as the newly-manufactured AveXis lots (Figure 35). In addition, the re-measurement of in vitro potency for lots AAV9SMN0613 and (b) (4) in March, 2019 (RPT-1333) found that the potency was close to 100% when the assay was performed with the correct amount of vector genomes. Together, these results make it unlikely that the decay of potency is meaningfully faster than the (b) (4) rate of decay in vg concentration.*

*The instability of DP cannot be explained by differences among lots in vector concentration, manufacturing method, final container or final formulation (b) (4). The instability is supported by three different assays (b) (4) in vitro potency and (b) (4) and a similar rate of decay in vg concentration is seen for (b) (4) DP. There is some uncertainty about whether the vg concentration is unstable when (b) (4) is stored frozen in (b) (4) containers, but there is no uncertainty that vg concentration is unstable for DP stored frozen in the final container.*

*The mechanism for instability is unknown, and we can do no more than speculate. Potential mechanisms include:*

- (b) (4)



The instability of DP raises two major issues. The first issue is determining the concentration of AAV9SMN0613 at the time that this lot was used in study CL-101. The second issue is determining an appropriate shelf life for DP.

#### Concentration of AAV9SMN0613

The vector genome concentration of AAV9SMN0613 was first measured by (b) (4) (SOP-137 v 3.0) in August, 2017, but the subjects in CL-101 were treated much earlier: between May, 2014 and December, 2015. The phase 3 studies began in December, 2017 using a dose ( $1.1 \times 10^{14}$  vg/kg) that was intended to be the same dose as in study CL-101. The original concentration of AAV9SMN0613 was  $1.96 \times 10^{13}$  vg/mL by (b) (4) at NCH, and the original dose in cohort 2 of CL-101 was  $2.0 \times 10^{14}$  vg/kg (using these (b) (4) units). The (b) (4) concentration of AAV9SMN0613 was measured at  $1.06 \times 10^{13}$  vg/mL in August, 2017. Based on the assumption that the vector is stable, the phase 3 dose was therefore adjusted to  $1.1 \times 10^{14}$  vg/kg (b) (4) units). This dose should be equivalent to  $2.0 \times 10^{14}$  vg/kg (NCH (b) (4) units), as long as the vector concentration is stable over time.

The data in amendment 53 indicate that the (b) (4) is not stable over time. The (b) (4) rate of decay can be projected backwards starting from the date that AAV9SMN0613 was first measured by (b) (4) (August, 2017). However, the uncertainty in the rate leads to considerable uncertainty when trying to calculate what the concentration would have been 2-3 years earlier (Figure 67).

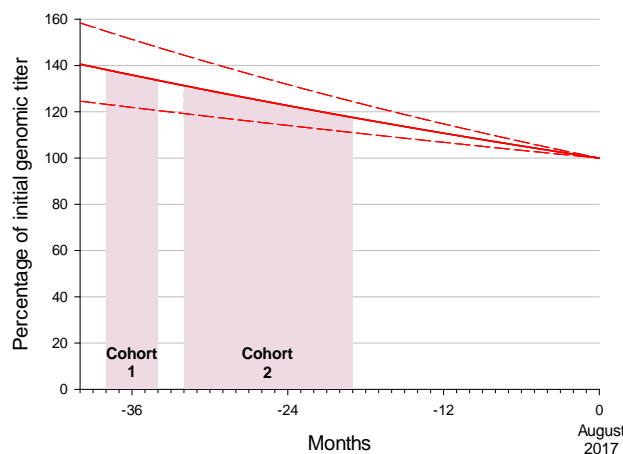


Figure 67 Retrospective estimation of the concentration of lot AAV9SMN0613 at the time that it was used in study CL-101. The 95% confidence intervals are indicated by dashed lines.

In addition to the uncertainty in the rate of decay, there is also uncertainty regarding the concentration of vector in August, 2017. The concentration was measured by (b) (4) in August, 2017 at  $1.06 \times 10^{13}$  vg/mL. However, the concentration can also be determined (possibly more accurately) from the AAV9SMN0613 regression line in Figure 61 – the concentration from the regression is  $9.65 \times 10^{13}$  vg/mL. The doses administered in CL-303 can also be adjusted in the same manner, but for CL-303 the adjustments are relatively small because all lots were administered within 6 months of the date of manufacture. Performing the adjustments in this manner suggests that the doses administered in cohort 2 of CL-101 had some overlap with the doses administered in CL-303, but the cohort 2 doses were higher overall (Figure 68). If, on the other hand, the concentration of AAV9SMN0613 on August, 2017 is assumed to be the concentration that was actually measured on that date ( $1.06 \times 10^{13}$  vg/mL), then the doses administered in cohort 2 of CL-101 become substantially higher than in CL-303 (Figure 69).

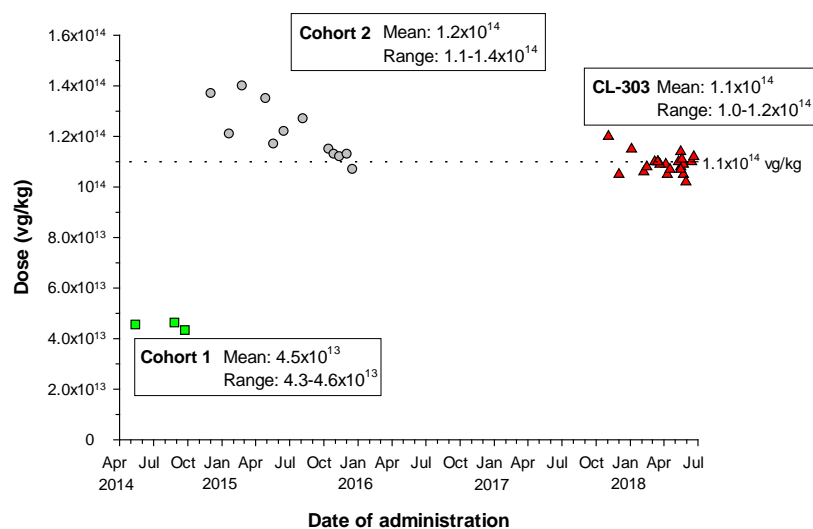


Figure 68 Estimated doses administered in studies CL-101 and CL-303. The adjustments assumed a vg decay rate of  $(b) (4)$  /year and a starting (August, 2017) concentration of  $9.65 \times 10^{13}$  vg/mL for lot AAV9SMN0613

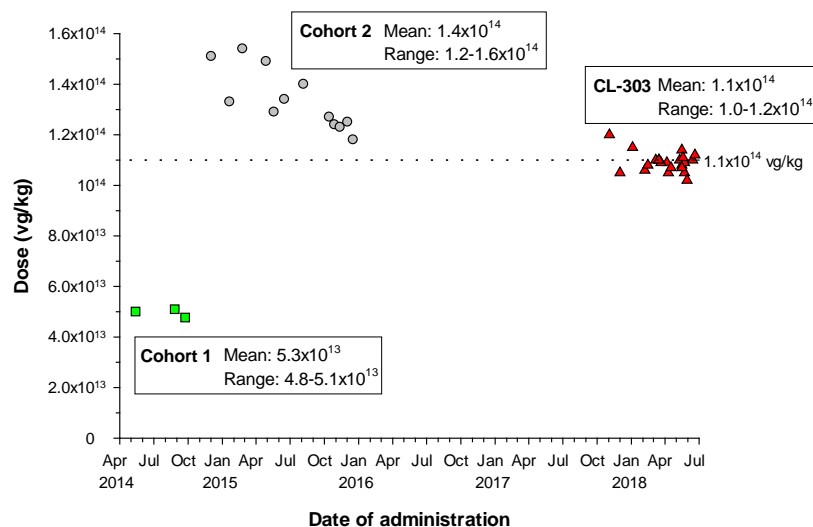


Figure 69 Estimated doses, based on a starting (August, 2017) concentration of  $1.06 \times 10^{13}$  vg/mL for lot AAV9SMN0613

*Reviewer comment: Small changes in assumptions about AAV9SMN0613 concentration – together with uncertainty in the decay rate – cause high uncertainty when trying to calculate the doses that were administered to subjects in study CL-101. It is very likely that the doses in cohort 2 of CL-101 were higher than the doses administered in CL-303. Differences in dose in this range could be clinically meaningful – mouse survival data show meaningful changes in survival when mice receive doses higher or lower than  $1.1 \times 10^{14}$  vg/kg (Figure 36).*

*The analysis in Figure 68 and Figure 69 suggests that the doses in cohort 2 may have averaged 9% to 27% higher than the intended dose of  $1.1 \times 10^{14}$  vg/kg. The range of doses administered in cohort 2 is wider, and for individual subjects in cohort 2 the dose may have extended to 27% to 45% higher than the intended dose. The width of the 95% CI was approximately  $\pm 10\%$  during the time when subjects in*

cohort 2 were being treated (Figure 67). It is important to note that these models are extrapolating the stability well beyond the real-time stability data – i.e. they make the assumption that the decay rate in the first year will continue to be similar in subsequent years. If this assumption does not hold, the model will not be accurate. Although these uncertainties make it impossible to determine the actual doses that were administered in cohort 2, a reasonable estimate is that the doses may have been up to (b) (4) higher than the intended dose of  $1.1 \times 10^{14}$  vg/kg.

Because the doses administered in CL-101 are uncertain and most likely higher than  $1.1 \times 10^{14}$  vg/kg, the outcomes of CL-101 cannot be relied upon to predict the safety and efficacy of the phase 3 / commercial dose of  $1.1 \times 10^{14}$  vg/kg. In contrast, we can be confident of the doses administered in CL-303 (Figure 68 and Figure 69), and the outcomes of CL-303 provide much better support for the commercial dose of  $1.1 \times 10^{14}$  vg/kg.

Regarding an appropriate shelf life for DP, the applicant will be adding a (b) (4) (lots manufactured after licensure will have a  $2.1 \times 10^{13}$  vg/mL target for DP concentration). More importantly, the minimum acceptance criterion for vg concentration has been raised to (b) (4) vg/mL for all lots. These changes will ensure that vg concentration of the commercial lots remains in a similar range to the concentration of lots used in study CL-303, throughout the full 12 month DP shelf life. Thus, the commercial dose of  $1.1 \times 10^{14}$  vg/kg will be the same as the dose administered in study CL-303.

#### *Stability of DP at 2-8°C*

The vector genome concentration of refrigerated DP was measured in a 6 month study (RPT-411), with no meaningful change over time (Figure 70). This stability at 2-8°C contrasts with the decline in vg concentration seen when the same lots were stored at  $\leq -60^\circ\text{C}$  (Figure 60). When a global fit to an exponential decay equation is performed (Figure 71), the rate of decline is not distinguishable from (b) (4) decline to (b) (4) increase per year).

(b) (4)

1 page determined to be not releasable: (b)(4)

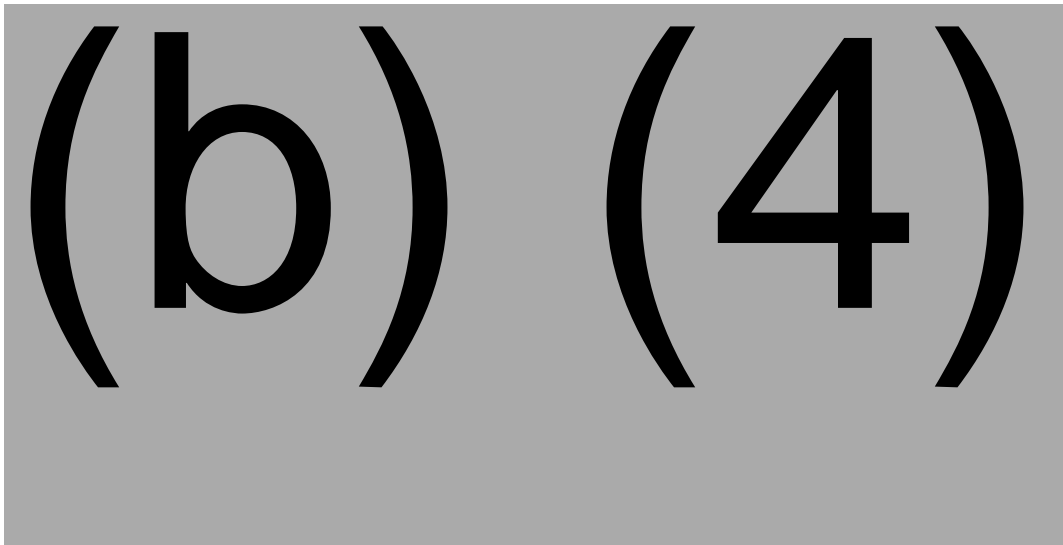


Figure 73 Stability of (b) (4) at 2-8°C

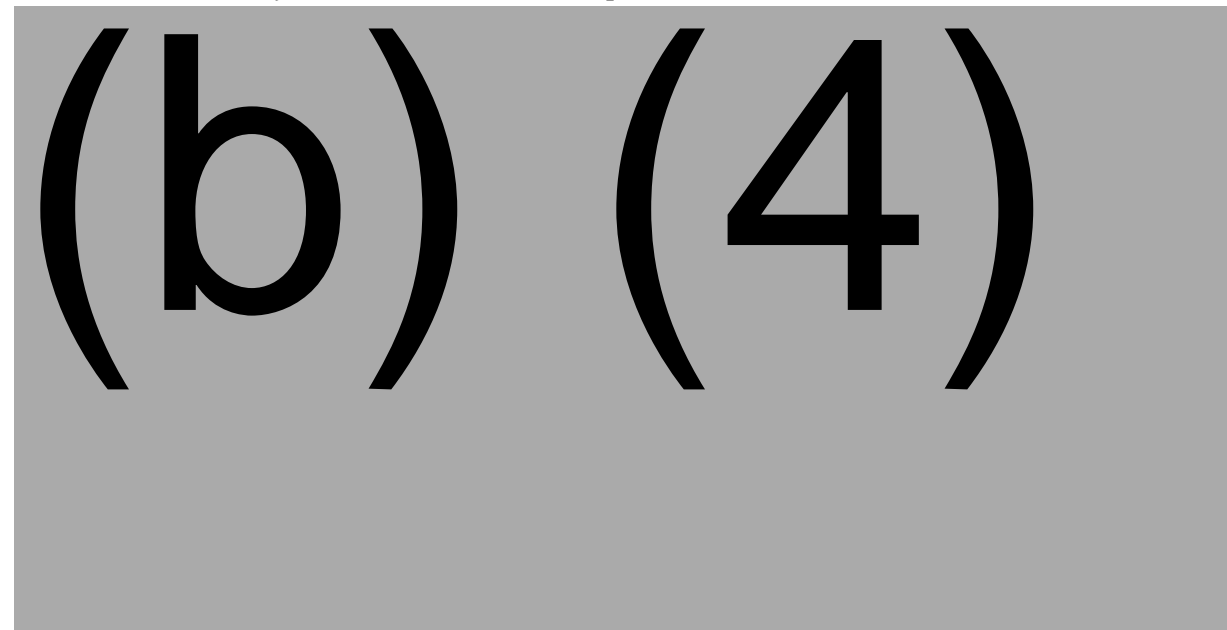
*Reviewer comment: DP is shipped to the site of administration on (b) (4) and then stored in a refrigerator for up to 14 days. The stability data above are sufficient to demonstrate the stability of genome concentration and potency for 14 days.*

*The greater stability of vg concentration when DP is refrigerated than when frozen suggests that the mechanisms of instability are different for refrigerated and frozen DP.*

#### *Stability of DP at room temperature*

The stability of lot (b) (4) was evaluated for 3 months (b) (4) and subsequent lots were evaluated for just one month. The (b) (4) (Figure 74) declined at a rate of (b) (4) per year, with very high uncertainty about the rate (b) (4). The potency declined by a substantial amount within one month (Figure 75) and (b) (4) showed variable results (Figure 73). In the (b) (4) impurity assay, the amount of impurities (degraded capsid proteins) increased over time during storage at (b) (4), but did not exceed (b) (4) (Figure 77).

A separate room temperature stability study was performed to demonstrate that DP is stable when held in the delivery device for 8 h at room temperature (3.2.P.2.6).



1 page determined to be not releasable: (b)(4)

(b) (4)

*Reviewer comment: Accelerated stability data indicate that (b) (4) purity is not very useful as a stability-indicating assay. The best stability assay is (b) (4) because of its high precision and accuracy. The in vitro potency assay is also an important assay to measure the stability of DP potency.*

*The (b) (4) study indicates that the vector vg concentration is unchanged by freezing. This finding suggests that the decline in vg concentration seen in the long-term  $\leq -60^{\circ}\text{C}$  studies is gradual, and is not due to any short-term damage that might occur during freezing of DP.*

### **3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment** (reviewed by AB)

The original BLA submission contained a plan to further evaluate the stability of DP at  $\leq -60^{\circ}\text{C}$  (Table 43). The (b) (4) DP lots that are already on stability will continue to be followed for at least (b) (4), and at least (b) (4) additional DP lot will be placed on stability (b) (4) and followed for at least the commercial shelf life. Updated stability information will be submitted in the annual report, including reporting of deviations and OOS results. If a DP lot on stability is confirmed to be OOS during the approved DP shelf life, the affected DP lot will be withdrawn and the Agency will be consulted.

No additional studies of DP stability are planned for the refrigerated temperature or room temperature.

(b) (4)

*Reviewer comment: During the March 28, 2019 late cycle meeting, we stated that we might be able to drop the (b) (4) assay from stability studies because it was redundant with the potency assay. However, the stability data submitted in amendment 53 on March 29, 2019 changed that perspective. It seems prudent to continue to include the (b) (4) in the stability program because this assay is not susceptible to problems with the reference standard vector in the way that the in vitro potency assay is.*

*During the May 2, 2019 teleconference, the applicant agreed to add the (b) (4) assay to future stability studies to evaluate the possibility of (b) (4) at the long-term storage temperature of  $\leq -60^{\circ}\text{C}$ . The (b) (4) assay was added (Table 43) in amendment 79 (May 10, 2019).*

**Overall Reviewer's Assessment of Section 3.2.P.8:**

When stored long-term at  $\leq -60^{\circ}\text{C}$ , DP is losing vector genomes at a rate of (b) (4) during the first year. Because there is currently only 1 year of real-time stability data, it is unknown whether the genome concentration will continue to decline at this rate in subsequent years. The potency and (b) (4) of DP are also definitely declining, although it is difficult to be precise about the rate of decline for potency and (b) (4) because of the variability of these assays and uncertainty about whether the assays might drift over time.

A (b) (4) loss is modest and is being managed with (b) (4), a tight lower limit for vg concentration, and a short shelf life. New lots manufactured under the license will include a (b) (4), and the minimum lot release acceptance criterion for the vg concentration was raised to (b) (4) vg/mL for all lots. Together, these changes will ensure that the vg



concentration remains above (b) (4) vg/mL for the full 12 month shelf life. The specification for vg concentration in the post-approval stability protocol is (b) (4) vg/mL.

The instability of DP and uncertainty about the long-term rate of decline create a serious problem when trying to determine the doses that were administered to subjects in study CL-101. Because the vg concentration of lot AAV9SMN0613 was measured by (b) (4) only 2 to 3 years after subjects in CL-101 were treated, the doses administered in study CL-101 were almost certainly higher than initially thought. The exact doses can only be roughly estimated because of uncertainty about the long-term rate of decline. FDA analysis indicates that the doses in study CL-101 may have been up to 40% higher than initially thought, meaning that the dose in cohort 2 of CL-101 may have been up to 40% higher than  $1.1 \times 10^{14}$  vg/kg.

The dose used in CL-303 was definitely  $1.1 \times 10^{14}$  vg/kg (even after taking into account the impact of instability), and this will be the licensed dose.

The current stability data do not adequately evaluate whether DP forms aggregates during long-term storage, or whether there are any other changes in capsid density distribution. In amendment 79 on May 10, 2019, the applicant added the (b) (4) assay to the DP stability protocol (Table 43).

### 3.2.A APPENDICES

#### 3.2.A.1 Facilities and Equipment

Reviewed by DMPQ

#### 3.2.A.2 Adventitious Agents Safety Evaluation (reviewed by AW)

The adventitious agent safety strategy consists of:

1. (b) (4)

#### Viral Clearance Studies

A viral clearance study 076-AVXS-101 was performed at (b) (4)



*The studies demonstrates that the manufacturing process will remove enveloped virus during the (b) (4) addition and the (b) (4) viruses will be removed during the (b) (4) steps. The viral inactivation and clearance studies are adequate.  
No major deficiencies in the Viral clearance studies*

**Overall Reviewer's Assessment of Section 3.2.A.2:**

The studies demonstrate that the manufacturing process will remove enveloped virus during the (b) (4) addition and the (b) (4) will be removed during the (b) (4) steps. The viral inactivation and clearance studies are adequate. No major deficiencies in the Viral clearance studies.

**3.2.A.3 Novel Excipients**

Not applicable

**3.2.R Regional Information** (Reviewed by AB)

**❑ Executed Batch Records**

This section of the BLA contains:

- Executed batch record 3325-01 from NCH, for the final (b) (4) step for lot AAV9SMN0613. (b) (4) were (b) (4) on (b) (4)
- The executed batch records from DS lot (b) (4) (manufactured (b) (4) and DP lot (b) (4) (manufactured (b) (4). DP lot (b) (4) was derived from DS lot (b) (4). This was the first manufacturing run immediately after the (b) (4) PPQ lots.
- The initial BLA submission contained MBRs for the AveXis manufacturing processes that were current at the time, but these MBRs have been updated throughout the review period. The MBRs do not contain any listing of the changes that occurred with each new version of the MBR, and therefore it was not clear whether there were any major process changes. In IR #32 we requested information on all of the MBR changes, and the applicant provided detailed information in amendment 37 (February 25, 2019). The only potentially major process change identified by FDA was a change from the (b) (4). The applicant has different MBRs for each (b) (4). We requested more information in IR #44, and in amendment 51 (March 22, 2019) the applicant explained that the two iCELLis systems were functionally the same, except for the user interface.
- AveXis has filled DP at multiple different volumes, and each of these fill volumes is associated with a different MBR. The current DP fill volumes are 5.5 mL and 8.3 mL. Lot (b) (4) was filled at (b) (4), and the PPQ lots were all filled at (b) (4) or (b) (4).

**❑ Method Validation Package**

This section of the BLA contains some of the validation reports for assays that are performed for release of (b) (4) DP. These validation reports are reviewed and discussed under sections 3.2.S.4.3 and 3.2.P.5.3.

**❑ Combination Products**

Not applicable

**❑ Comparability Protocols**

The applicant does not propose any future manufacturing changes that will be evaluated under a comparability protocol.

## Other eCTD Modules

### Module 1

#### A. Environmental Assessment or Claim of Categorical Exclusion (Reviewed by AB)

The applicant's environmental assessment is provided in 1.12.14, in accordance with 21 CFR 25. This application is not eligible for categorical exclusion, and the applicant does not make a claim of categorical exclusion. The applicant does not propose any alternative action other than approval.

The product onasemnogene abeparvovec-xioi is derived from AAV9, a nonpathogenic human DNA virus that is incapable of autonomous replication. In this product, the (b) (4) of AAV9 has been (b) (4). The product is capable of a (b) (4)

The manufacturing process is designed to minimize the potential that (b) (4)

Even if (b) (4)

The product is manufactured using (b) (4), and therefore carries a theoretical risk of being contaminated with adventitious agents (viruses or bacteria). The biological starting materials (b) (4) are tested to ensure absence of adventitious agents, and each lot of product also undergoes in-process testing to ensure absence of adventitious agents. The manufacturing process is also validated to remove or inactivate model viruses.

This product will be administered at hospitals or treatment centers using universal precautions, and unused product and product-contact materials will be disposed of as biohazardous medical waste. The product is relatively stable (compared to other viruses) at room temperature, but will degrade over time into naturally-occurring materials. The applicant estimates that up to 260 patients will receive the product each year in the US.

Data from a clinical study demonstrate that patients who are treated with onasemnogene abeparvovec-xioi will shed vector DNA in stool for 1-2 months. DNA will also be shed in saliva and urine at lower levels and for shorter periods of time after administration. It is not known how much of the shed DNA is (b) (4), as opposed to shedding of naked DNA. Even if (b) (4), the risk of causing infectious disease is zero because the product is inherently incapable of causing infectious disease, and there will be no direct toxic effects from exposure to small amounts of this vector, even if it is intact.

*Reviewer comment: The Agency concludes that there will be no significant environmental impact from approval of onasemnogene abeparvovec-xioi, and a finding of no significant impact (FONSI) will be prepared.*

#### B. Labeling Review (reviewed by AB)

##### Full Prescribing Information (PI):

##### Sections 2 (Dose and Administration) and 3 (Dosage Forms and Strengths)

The product is supplied frozen at a nominal concentration of  $2.0 \times 10^{13}$  vg/mL in 10 mL vials with fill volumes of either 5.5 mL or 8.3 mL. Based on the patient's weight, the appropriate number of vials are assembled into a kit. Each of the possible kits has a separate NDC number. The recommended dose is  $1.1 \times 10^{14}$  vg/kg, administered as a single i.v. infusion over 60 minutes. Before use, the product is thawed and the appropriate volume collected in a syringe. The PI states that the syringe should be discarded if not used within 8 h, and stability for 8 h at room temperature is supported by studies in 3.2.P.2.6.

In the original submission, the applicant proposed restricting the patient weight range to 2.6 (b) (4) kg, with the drug to be supplied as a weight-specific kit consisting of up to (b) (4) total vials with volumes of

either 5.5 mL or 8.3 mL. A 5.5 mL vial is sufficient for 1 kg of body weight, and an 8.3 mL vial is sufficient for 1.5 kg of body weight. The patient will receive the entire kit contents, meaning that patients at the low end of each half-kg weight range will receive a slightly higher dose than patients at the high end of each half-kg weight range. For example, a patient weighing 3.1 kg will receive the same 19.3 mL kit as a patient weighing 3.5 kg. Therefore, the patient weighing 3.1 kg will receive a dose of  $1.24 \times 10^{14}$  vg/kg, and the patient weighing 3.5 kg will receive a dose of  $1.1 \times 10^{14}$  vg/kg.

During the course of the review, FDA requested that the range of kits be expanded to accommodate heavier patients, and as a result the final range of kit sizes will accommodate patients weighing from 2.6 kg to 13.5 kg (2 to 9 vials, in the same carton as before). The PI will state that patients weighing more than 13.5 kg will require (b) (4). The shipping validation was performed again for the 9-vial kit (RPT-921, amendment 50, March 22, 2019).

In amendment 63 (April 12, 2019), the applicant provided a study (RPT-1328) showing that the thaw time for the kit is 12 h when refrigerated or 4 h at room temperature. This study was performed at the maximum kit volume (9 vials, each filled with 8.3 mL of (b) (4), plus 9 alcohol swabs). Vials were observed (b) (4)

### **Section 11 (Description)**

ZOLGENSMA (onasemnogene abeparvovec-xioi) is a suspension of an adeno-associated viral vector-based gene therapy for intravenous infusion. It is a recombinant self-complementary AAV9 containing a transgene encoding the human survival motor neuron (SMN) protein, under the control of a cytomegalovirus enhancer/chicken- $\beta$ -actin-hybrid promoter.

ZOLGENSMA has a nominal concentration of  $2.0 \times 10^{13}$  vg/mL. Each vial contains an extractable volume of not less than either 5.5 mL or 8.3 mL and the excipients 20 mM tris (pH 8.0), 1 mM magnesium chloride ( $\text{MgCl}_2$ ), 200 mM sodium chloride (NaCl) and 0.005% poloxamer 188. ZOLGENSMA is sterile and contains no preservative.

### **Section 12 (Clinical Pharmacology)**

The shedding studies are described in section 12.3 of the PI, based on review of shedding data from 5 subjects in CL-101 (see review of module 5 later in this review). Vector DNA was evaluated in saliva, urine and stool. In line with FDA guidance, vector infectivity was not evaluated. Vector DNA was shed for 1-2 months in stool (negative by 2 months), and at much lower levels in urine and saliva for a few weeks.

This section of the PI also contains a description of the biodistribution of vector and SMN protein in two patients who died after receiving onasemnogene abeparvovec-xioi. The assays used in the animal and human biodistribution studies are reviewed below in the sections for module 4/5.

### **Section 14 (Clinical Studies)**

Due to considerable uncertainty about the doses that were administered in study CL-101, the primary evidence for efficacy is being provided by study CL-303.

Information about study CL-101 in the NCT database (NCT02122952) and in Mendell et al. (2017), NEJM 377:1713 indicates that the doses in study CL-101 were  $6.7 \times 10^{13}$  vg/kg (for cohort 1) and  $2.0 \times 10^{14}$  vg/kg (for cohort 2). These doses were based on concentrations determined using an inaccurate and imprecise qPCR assay from NCH. Concentrations determined by the new AveXis (b) (4) assay in August, 2017 for lot AAV9SMN0613 were used to revise the doses to  $3.7 \times 10^{13}$  vg/kg and  $1.1 \times 10^{14}$  vg/kg. As discussed in 3.2.P.8, our best estimate (which still has a high degree of uncertainty) is that the doses administered in study CL-101 may have been up to 40% higher than the intended doses of  $3.7 \times 10^{13}$  vg/kg

and  $1.1 \times 10^{14}$  vg/kg. When discussing the doses in study CL-101, the PI communicates the change in dose units, the uncertainty about the doses, and the likelihood that the doses in CL-101 were substantially higher than originally thought.

### ***Section 16 (How supplied / storage and handling)***

The product is supplied in a kit of 2-9 vials, packaged into a carton. The number of vials and the vial volumes depend on the weight of the patient, and there is a kit for every half-kg of weight between 2.6 and 13.5 kg, and each kit size has a separate NDC number. Each kit also contains one alcohol wipe per vial. When an order is placed, an appropriately-sized kit is assembled into a carton and shipped on (b) (4).

When the kit is received, it should be placed in a refrigerator, where it is stable for up to 14 days. The carton contains text stating “must use within 14 days of receipt,” and the carton variable label includes a large blank space to write the date of receipt. The expiration date listed on the variable label is the nearest expiration date of the vials in the kit, not the expiration date of the kit itself, which will always be 14 days after receipt. The kit may contain vials from multiple different lots (for example, 5.5 mL and 8.3 mL vials will always be from different lots).

*Reviewer comment: This product is provided in a novel kit form based on the weight of the patient. Patients will receive a dose equal to or slightly greater than the recommended  $1.1 \times 10^{14}$  vg/kg dose. For example, a patient weighing 3.1 kg will receive the kit for 3.1-3.5 kg that contains 19.3 mL, for a dose of (b) (4) vg/kg (uncorrected for instability). The same method of dosing was used in study CL-303.*

*The PI contains adequate instructions for thawing and storage of the kit in a refrigerator, with appropriate instructions to use the kit within 14 days of receipt, and to use syringes within 8 h of loading the product into syringes.*

*The descriptions of shedding and biodistribution studies in the PI are based on sound methodology.*

### **Carton and Container Label:**

The product is in 10 mL containers with either a 5.5 mL or 8.3 mL fill volume. All containers have green caps. The initial container label was not acceptable because there was a green dot on the 5.5 mL vial that was the same color as the green caps. The container labels were updated in amendment 43 (February 26, 2019) to include grey dots for the 5.5 mL volume and purple dots for the 8.3 mL volume. The container and package labels were updated in response to IR #48 in amendment 64 (April 17, 2019) to correct several mistakes. The container and package labels were updated again in response to IR #55 in amendment 72 (May 2, 2019) to correct several issues that were not in compliance with 21 CFR 610.62. The applicant submitted amendment 89 (May 20, 2019) to add the license number to the vial labels, to make a minor change to the refrigerated temperature range (lower limit changed from (b) (4) 36°F, with no change to the lower limit of 2°C) and to change the item number on the carton from 1674 to 1729.

The container labels (Figure 78 and Figure 79) contain all required text. The final container labels were received in amendment 89 (May 20, 2019). The lot number and expiration date will be printed as variable text in the lower right hand corner. There is no requirement to include the product concentration or strength on the vial label (the package artwork indicates that the strength is  $2.0 \times 10^{13}$  vector genomes/mL). The proper name is at least as prominent as the tradename.

The carton artwork (Figure 80) includes the phrase “must use within 14 days of receipt,” which is in accordance with the language in the package insert. This final carton artwork was received in amendment 83 (May 15, 2019).

Variable information for the carton is on the carton variable label in Figure 81 (lot number for the kit, number of vials, expiration date, NDC number, etc.). The final carton variable label was received in amendment 80 (May 13, 2019).

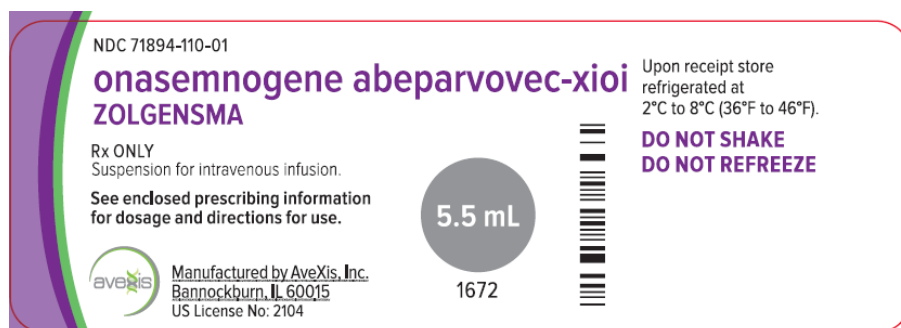


Figure 78 Vial label for 5.5 mL fill volume

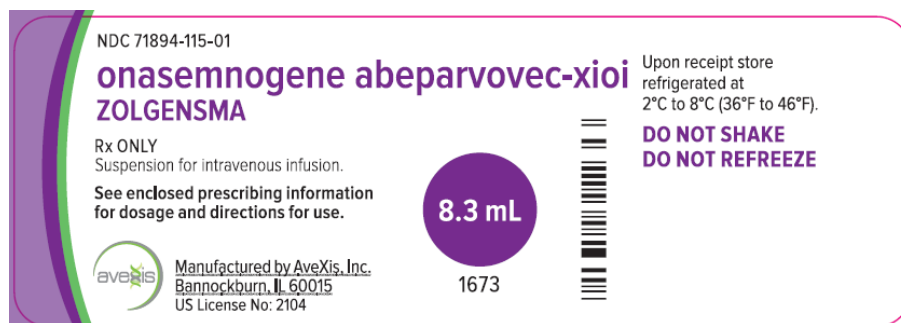


Figure 79 Vial label for 8.3 mL fill volume

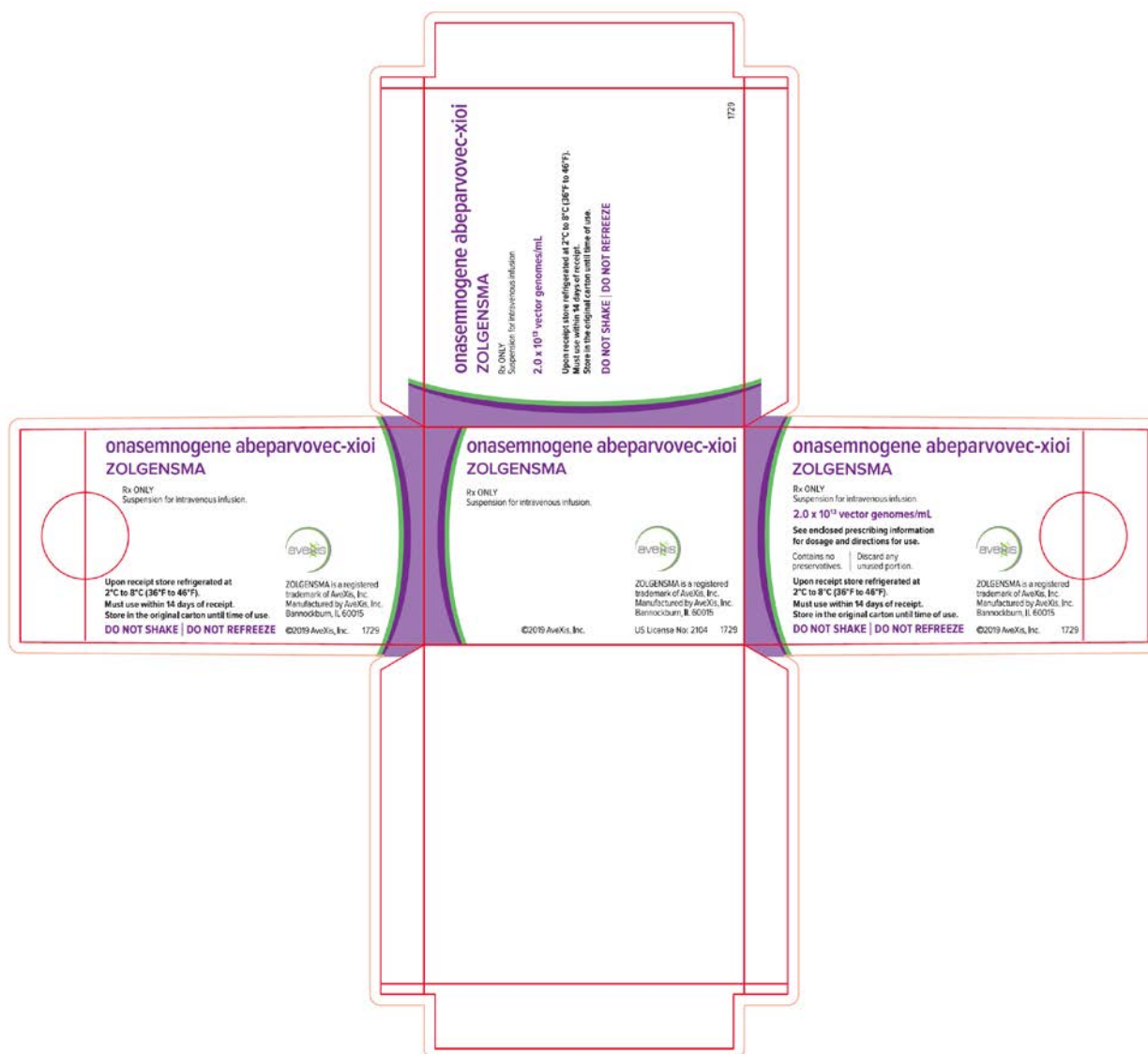


Figure 80 Carton artwork

Each carton (“kit”) will contain 2-9 vials, and every kit will be assigned a unique lot number by AveXis that is traceable to the lot numbers of the vials in the kits. This information is printed on a variable kit label that is placed on the carton (Figure 81). There are multiple different kit labels for the multiple different kit sizes, and each kit size has its own NDC number. The expiration date on the kit label refers to the earliest expiration date on the vials within (the vials may be from multiple different lots).

Vials can only be shipped if they have at least 14 days of shelf life remaining, because the kit can be stored refrigerated for up to 14 days after receipt. There is a blank space on the variable label for the recipient to write the date of receipt. In amendment 80 (March 13, 2019), the applicant stated that they will not distribute vials that have less than 60 days of shelf life remaining. However, in amendment 83 (March 15, 2019), the applicant submitted revised carton artwork that states “must use within 14 days of receipt.” This new language agrees with the final language in the package insert, and it is now acceptable for the applicant to distribute vials as long as they have at least 14 days of shelf life remaining.



	<b>Patient Weight:</b> 2.6 – 3.0 kg
	<b>Kit Contents:</b> 8.3mL vial x 2 Alcohol Wipe x 2 Kit Item 3
	<b>onasemnogene abeparvovec – xioi</b> <b>ZOLGENSMA      NDC 71894 – 130 – 02</b>
	<b>Date of Receipt:</b> <div style="border: 1px solid black; width: 180px; height: 50px; display: flex; align-items: center; justify-content: center;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">           GTIN 0037189413002            SN AX113018008            EXP 14DEC2020            LOT (b) (4)         </div>  </div>

Figure 81 Variable label for carton

*Reviewer comment: vial labels, package labels and variable labels for the package are acceptable.*

## **Modules 4 and 5**

### **Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints** (Reviewed by AB)

#### **Anti-AAV9 antibody ELISA**

Information on the anti-AAV9 ELISA is contained in both the original BLA submission and in amendment 10. The assay is a laboratory-developed test (LDT). The draft PI contains the following statement: “The safety and efficacy of ZOLGENSMA in patients with anti-AAV9 antibody titers above 1:50 have not been established. Patients should be tested for the presence of anti-AAV9 antibodies prior to infusion with ZOLGENSMA.”

(b) (4)

[REDACTED]

In RPT-302, (b) (4) validated the assay for ability to detect the positive and negative controls. Intermediate precision (OD values) did not exceed (b) (4) CV. Background (no-antigen) levels averaged (b) (4). In amendment 10, the applicant states that the same positive and negative control samples were run in both the NCH assay and the (b) (4) assay to establish reproducibility. Less information is provided on the (b) (4) assay, but the applicant states that identical samples were run at both (b) (4), and (b) (4) of samples yielded concordant qualitative results between the two test facilities thereby

providing assurance that testing performed at either site will provide similar results.” The applicant did not, however, provide the reproducibility data.

During CL-101, one subject was excluded from CL-101 due to antibody titer exceeding 1:50, and no subjects with a titer above 1:50 were administered the product. At least one subject had a titer exceeding the cutoff at initial screening, but this subject later screened negative after cessation of breast feeding and was successfully treated with vector. All subjects became positive (>1:50) for anti-AAV9 antibodies by one month after treatment, and some were positive as soon as 1 week after treatment.

*Reviewer comment: The anti-AAV9 ELISA is fit for purpose. However, the performance characteristics of this assay cannot be fully evaluated due to the design of the assay (lack of a quantitative (b) (4) standard, lack of information about the (b) (4) assay) and, more importantly, absence of clinical information about the impact of pre-existing antibodies that would be necessary to support an appropriate cutoff.*

*The lack of a (b) (4) standard renders the assay vulnerable to upward or downward shifts in (b) (4) caused by changes in (b) (4). It is likely that borderline-positive samples will shift up to (b) (4), and therefore the assay likely does not have good ability to discriminate between a “negative” sample with a (b) (4) and a (b) (4) sample with a (b) (4). However, this is a minor concern in the current situation because of the lack of clinical information about an appropriate cutoff: it is not clear whether a change from 1:50 (b) (4) is meaningful. Although animal studies indicate that the presence of anti-AAV9 antibodies will have a potentially severe negative impact on efficacy of AAV9 vectors, it is unclear how to translate the animal data into an appropriate cutoff.*

*In conclusion, the assay is fit for purpose, but the accuracy of the results for borderline-positive samples is unknown. Because it has not been possible to establish a clinically-appropriate assay cutoff, it is acceptable for this assay to be used as an LDT while further information is gathered. It is reasonable to recommend testing of patients and to inform them that the safety and efficacy are unknown in patients with an anti-AAV9 antibody titer above 1:50, but it is not currently appropriate to restrict administration of the product based on these antibody results.*

(b) (4)

#### **Assays for vector DNA shedding and biodistribution in patients**

For PK and shedding studies in humans, the (b) (4) assay (SOP-137) was transferred to (b) (4) and validated for lack of interference from various matrices: urine, (b) (4) stool and (b) (4) saliva (RPT-270).

(b) (4)

The five heaviest subjects from the CL-101 high dose cohort had samples collected for shedding studies (5.3.4.2, RPT-270). The report does not state whether the remaining subjects had samples collected. Large amounts of vector DNA was detected in stool at early time points, with the highest amounts detected on day 1 ( $10^{10}$  vg/g of stool). The amount of vector DNA in stool declined over time and reached baseline ( $10^6$  vg/g of stool) by 1-2 months (Figure 82). Shedding in saliva and urine were much lower than in stool. Shedding in saliva was maximal at day 1 ( $10^8$  vg/mL) and declined to LOD by 3 weeks. Shedding in urine was maximal at day 1 ( $10^6$  vg/mL) and declined to baseline by 1-2 weeks. Because only vector DNA was measured, the amount of functional vector shed (if any) is unknown.

Thus, the major route of shedding was stool. Based on the initial dose of  $1.1 \times 10^{11}$  vg/g body weight, the maximum concentration of vector genome per g of stool was at least 10% of the predicted maximum tissue concentration of vector (Figure 83). Note that this does not mean that 10% of the total vector dose was shed in the stool. Subject (b) (6) had shedding in stool that was higher (on a per gram basis) than the initial dose. This does not mean that the vector was replicating: only that vector became relatively more concentrated in stool than in tissue.

Biodistribution studies were conducted in two patients who died after receiving onasemnogene-abeparvovec-xioi at the  $1.1 \times 10^{14}$  vg/kg dose in phase 3 studies. The first patient died 5.5 months after treatment, and a full autopsy was conducted. The cause of death was disease-related respiratory failure, and the autopsy did not discover any vector-related toxicity. Prior to death, the patient had shown improved CHOP-INTEND scores. The biodistribution report RPT-952 was submitted in amendment 35. In amendment 54, the RPT-952 was modified to include more information about the (b) (4) and to explain how they are specific for the vector genetic information and not for *SMN1* or *SMN2* gene or RNA sequences. For more accurate measurement of (b) (4) assay was modified to include a multiplex evaluation of the (b) (4) number as a reference.

Vector DNA was found throughout all levels of the spinal cord in amounts of (b) (4), and within the CNS in amounts of (b) (4) to (b) (4). Vector was found in all other organs tested, notably liver (several hundred copies per cell), spleen (b) (4) cell, lymph node (b) (4) cell and heart (b) (4) cell). Vector RNA was evaluated by non-quantitative (b) (4), and was positive in many tissues. Robust levels of SMN protein were detected in many organs by (b) (4), including in apparent spinal motor neurons.

A second patient died 1.7 months after treatment, and similar widespread biodistribution was seen for vector DNA and SMN protein (RPT-1342, amendment 70, April 30, 2019). *SMN* RNA was not measured because the RNA quality was poor by the time that samples were collected. An autopsy is pending.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

#### **Genetic assays for SMN1 and SMN2 genes in humans**

No information was provided in the BLA. Genetic assays for SMN1 and SMN2 are currently available as LDTs, and these assays aid in the diagnosis and management of patients with SMA. Newborn screening assays are also currently being rolled out at the state level. The PI will mention that all patients in CL-101 had two copies of SMN2, but the SMN2 copy number will not be listed in the indication because it is not completely predictive of SMA severity.

*Reviewer comment: CDER approved nusinersen without requiring an FDA-regulated genetic test for SMA. Similarly, we will not require an FDA-regulated genetic test before approval of onasemnogene abeparvovec-xioi.*

#### **Overall Reviewer's Assessment of Relevant Sections of Module 4 and 5:**

The assays used to analyze clinical and preclinical samples are adequately validated and fit for purpose.

- The anti-AAV9 antibody ELISA will be performed at CLIA-certified laboratories as a LDT. There are insufficient clinical data to determine whether the anti-AAV9 antibody cutoff used in clinical studies is appropriate, since all subjects enrolled had titers  $\leq 1:50$ . In its current form (without a calibration curve) the assay is best regarded as a semi-quantitative assay. All subjects became positive for anti-AAV9 antibodies after treatment with vector.
- The assay for anti-SMN antibodies is fit for purpose. All subjects were negative, before and after administration of the product.
- The assays for anti-AAV9 and anti-SMN antibodies are fit for purpose. All subjects became positive for T cells against AAV9 following treatment with vector, but none became positive for T cells against SMN.
- Vector DNA and RNA in human and mouse samples were detected with modified versions of the (b) (4) assay. The assays are fit for purpose. The major route of shedding in humans was

via stool, with maximal shedding occurring on day 1 after receipt of vector. The assays used only measure (b) (4) – they do not allow conclusions regarding whether shed vector genomes are still potent.

- The genetic assays used to aid in diagnosis of SMA were not submitted to the BLA, and were not reviewed.