EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

26 March 2020
EMA/200482/2020
Committee for Medicinal Products for Human Use (CHMP)
Committee for Advanced Therapies (CAT)

Assessment report

Zolgensma

International non-proprietary name: onasemnogene abeparvovec

Procedure No. EMEA/H/C/004750/0000

Note

The CAT Assessment report has been endorsed by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

Abbreviation	Definition
AAV	adeno-associated virus
AAV9	adeno-associated virus serotype 9
ACTIVE-mini	Ability Captured Through Interactive Video Evaluation – mini
ADaM	Analysis Data Model
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ASO	antisense oligonucleotide
BiPAP	bi-level positive airway pressure
CDISC	Clinical Data Interchange Standards Consortium
CHOP-INTEND	Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CMAP	compound motor action potential
CMV	cytomegalovirus
CSF	cerebrospinal fluid
CSR	clinical study report
ddPCR	droplet digital polymerase chain reaction
DILI	drug-induced liver injury
DSMB	Data Safety Monitoring Board
ECAS	efficacy completer analysis set
ECG	electrocardiogram
EES	efficacy evaluable set
EIM	electrical impedance myography
EMA	European Medicines Agency
EU	European Union
FAS	full analysis set
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
ICD-10	10th Revision of the International Classification of Diseases and Related Health Problems
ICH	International Council for Harmonization
IQR	interquartile range
IRB	Institutional Review. Board Inspections
IT	intrathecal
ITR	inverted terminal repeats
ITT	intent-to-treat
IV	intravenous
LOCF	last observation carried forward
LS	least squares
Max	maximum
Min	minimum
mITT	modified intent-to-treat
MMRM	mixed model repeated measure
mRNA	messenger ribonucleic acid
MUNE	motor unit number estimation
NA	not applicable
NAV	not available
NCH	Nationwide Children's Hospital
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NIV	non-invasive ventilation
OMIM	Online Mendelian Inheritance in Man
PI	principal investigator
PNCR	Pediatric Neuromuscular Clinical Research
qPCR	quantitative polymerase chain reaction
SAP	statistical analysis plan
SDTM	Study Data Tabulation Module
SMA	spinal muscular atrophy

SMN	survival motor neuron
SMN1	survival motor neuron 1
SMN2	survival motor neuron 2
SMN Δ7	delta 7 mouse model of SMA disease
SmPC	Summary of Product Characteristics
SS	safety analysis set
TBD	to be determined
US	United States
USPI	United States prescribing information
vg	vector genome
WHO	World Health Organization
WHO-MGRS	The World Health Organization Multicentre Growth Reference Study

1. Background information on the procedure

1.1. Submission of the dossier

The applicant AveXis EU limited, submitted on 9 October 2018 an application for marketing authorisation to the European Medicines Agency (EMA) for Zolgensma, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

Zolgensma, was designated as an orphan medicinal product EU/3/15/1509 on 19 June 2015 in the following condition: Treatment of spinal muscular atrophy.

Zolgensma was granted eligibility to PRIME on 26 January 2017 in the following indication: Treatment of paediatric patients diagnosed with spinal muscular atrophy (SMA) Type 1.

Eligibility to PRIME was granted at the time in view of the following:

- SMA is a devastating condition for which there was no approved therapy at the time PRIME eligibility was granted. Treatment options were palliative and limited to nutritional and ventilatory support, with management focused on pulmonary complications and orthopaedic care.
- The non-clinical programme had yielded results supportive of the expected mechanism of action with positive effects demonstrated in mice on survival, body weight, righting behaviour, and improved cardiac function.
- Additionally, it had been demonstrated that the vector had the ability to cross the blood brain barrier in two species (mice and cynomolgus Macaques) following IV administration.
- The preliminary clinical observations following Zolgensma's administration include positive impact on survival, pulmonary function, nutritional support, preservation of motor function and the attainment of development milestones, all of which were unexpected within the framework of the natural history and disease progression for SMA Type 1. These clinically meaningful responses in the patients treated with Zolgensma were sufficient preliminary clinical evidence of treatment effect that had the potential to address an unmet need in this devastating paediatric disease.

The applicant applied for the following indication: ZOLGENSMA is indicated for a single treatment of spinal muscular atrophy (SMA) Type 1. The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that onasemnogene abeparvovec was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Zolgensma as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/Zolgensma

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0162/2019 on the agreement of a paediatric investigation plan (PIP)

At the time of submission of the application, the PIP P/0162/2019 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Applicant's request(s) for consideration

Conditional marketing authorisation and Accelerated assessment

The applicant requested consideration of its application for a conditional marketing authorisation in accordance with Article 14-a of the above-mentioned Regulation.

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

New active Substance status

The applicant requested the active substance onasemnogene abeparvovec contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

PRIME support

Upon granting of eligibility to PRIME, Johannes Hendrikus Ovelgönne was appointed by the CHMP as Rapporteur.

A kick-off meeting was held on 15 May 2017. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures: changes to manufacturing process, potential studies on nonclinical model to investigate different doses and routes of administration, target population and proposed indication, route of administration and choice of dose, maintenance of the effect and retreatment, plans for post-authorisation long-term follow-up of efficacy and safety and regulatory aspects.

Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the subject to the present application:

Date	Reference	SAWP co-ordinators
26 January 2017	(EMEA/H/SA/3421/1/2016/PA/PED/SME/ADT/II)	Dr Caroline Auriche, Dr Armando Magrelli
14 December 2017	(EMEA/H/SA/3421/1/FU/1/2017/PA/PED/SME/ADT/PR/II)	Dr Hans Ovelgönne,

Date	Reference	SAWP co-ordinators
		Dr Rune Kjeken Dr Armando Magrelli
25 January 2018	(EMEA/H/SA/3421/1/FU/2/2017/ PA/PED/SME/ADT/PR/II).	Dr Hans Ovelgönne Dr Rune Kjeken

The initial and follow-up advice in 2017 concerned clinical questions while in 2018, the applicant asked questions related to the quality and pre-clinical development.

The Protocol assistance pertained to the following clinical aspects:

- Acceptability of the design of the open-label, single-arm, single-dose, historical-controlled, multi-centre Phase 3 study in Europe to support a MAA filing for the treatment of SMA Type 1 regarding efficacy and safety.
- Agreement that the initial marketing authorisation would be based on data from newborn infants and infants with SMA Type 1, who at time of initial gene therapy would be six months of age or younger as well as on the overall clinical development program.
- Further, it was discussed if the design of the proposed global multi-center phase 2/3 open label, natural-history controlled protocol, involving a single, one-time dose of Zolgensma delivered intravenously to infants with genetically diagnosed and pre-symptomatic SMA with multiple copies of *SMN2* (Protocol AVXS-101-CL-304) would be acceptable to support an MAA in a broad SMA indication. The design aspects discussed pertained to the historical control, patient population, sample size, primary and secondary endpoints and the approach for statistical analysis and dose.
- The applicant also asked whether the Agency agreed that assuming the results from a comparability assessment between the Phase 1 and Phase 3 product would be acceptable, if the product could meet the requirements for a marketing authorisation with the ongoing trials as a post authorisation commitment or a conditional marketing authorisation.

Quality and pre-clinical:

- In 2018, the comparability protocol approach to link the product used in the Phase 1 study with the product intended to be used in the Phase 3 study and in the proposed commercial process was discussed. Specific questions were asked regarding the list of attributes, acceptance criteria, the proposed manufacturing and testing approach, stability program for active substance and finished product, shelf-life, quantitative potency assay development and qualification plan, release testing.
- The non-clinical question concerned the overall non-clinical development. There was also a multidisciplinary question asking if the Agency agreed that the comparability approach which will be available at time of the MAA submission, could be sufficient to support the quality part of the MAA.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Johannes Hendrikus Ovelgonne Co-Rapporteur: Egbert Flory

For the appointed rapporteur it was considered exceptionally justified that the individual had previously been acting as coordinator for Scientific Advice on the development relevant for the indication subject to the present application.

The application was received by the EMA on	9 October 2018
Accelerated Assessment procedure was agreed-upon by CAT and CHMP on	26 July 2018
The procedure started on	1 November 2018
The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	22 January 2019
The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	24 January 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	5 February 2019
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	14 February 2019
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 February 2019
The applicant submitted the responses to the CAT consolidated List of Questions on	25 April 2019
The following GMP and GCP inspections were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection at one investigator site and at the sponsor site in USA was conducted between 10/12/2018 and 18/01/2019. The outcome of the inspection carried out was issued on.	14 February 2019
– A GMP inspection at one site responsible for manufacture of the active substance and finished product in the USA between 28/01/2019-01/02/2019. The outcome of the inspection carried out was issued on.	14 June 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	6 June 2019
The CAT agreed on a list of outstanding issues in writing to be sent to the applicant on	21 June 2019
A SAG was convened to address questions raised by the CHMP on The CAT and CHMP considered the views of the SAG as presented in the minutes of this meeting.	6 September 2019

The applicant submitted the responses to the CAT List of Outstanding Issues on	18 October 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	22 November 2019
The CAT agreed on a 2nd list of outstanding issues in writing to be sent to the applicant on	06 December 2019
The applicant submitted the responses to the CAT List of Outstanding Issues on	23 January 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	5 March 2020
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Zolgensma on	20 March 2020
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Zolgensma on	26 March 2020
The CAT and CHMP adopted a report on similarity of Zolgensma with Spinraza on (Appendix 1)	20-26 March 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The claimed therapeutic indication is treatment of (5q) spinal muscular atrophy (SMA) Type 1.

The term SMA is applied to a diverse group of genetic disorders, all of which affect the spinal motor neuron. The most common form of SMA results from bi-allelic mutations to the survival motor neuron 1 (*SMN1*) gene on chromosome 5q13 (5q SMA) of which the majority are homozygous deletions.

5q SMA is conventionally classified into four phenotypes on the basis of age of onset and highest motor function achieved, with an additional phenotype (type 0) to describe the severe forms of antenatal-onset spinal muscular atrophy.

5q SMA Type 1 patients never attain independent sitting and have onset of symptoms within the first 6 months of life.

The clinical studies to support the indication focused on a homogeneous cohort of patients with unequivocal SMA "Type 1", based on the combination of clinical diagnosis (symptom onset prior to 6 months of age, hypotonia) and genotype (all enrolled patients were confirmed to have bi-allelic *SMN1* gene deletions and only 2 *SMN2* copies without the known *SMN2* genetic modifier).

2.1.2. Epidemiology

SMA (5q) is an autosomal recessive, early childhood disease with an incidence of approximately 1: 10,000 live births, of which approximately 45-60% of cases are SMA Type 1, the most severe form. Spinal muscular atrophy is the leading cause of infant mortality due to genetic disease.

There are limited treatment options for patients with SMA. Until recently, no causal treatment existed. Complications derived by SMA caused muscle weakness were addressed by multidisciplinary medical and supportive care, including orthopedic and spinal management, nutritional, swallowing and gastrointestinal management, pulmonary management, acute care, and palliative care. The 2007 published consensus statement for standard of care in SMA (Wang et al., J Child Neurol, 2007) was updated in 2017 due to improvements in many aspects of care for infants in children with SMA (Mercuri et al., Neuromusc Dis, 2018; Finkel et al, Neuromusc. Dis, 2018).

On the basis of available newborn screening, in 2018 a treatment algorithm for infants diagnosed with 5q SMA through Newborn Screening has been published (Glascock et al., J Neuromusc Dis, 2018), however, newborn screening for 5q SMA is not an implemented standard throughout Europe yet. Initiation of treatment is recommended even for pre-symptomatic 5q SMA patients that by genotype are likely to develop 5q SMA Types 1 or 2.

An approved causal treatment has become available only recently, when in late 2016 (23 December) and mid-2017, the United States Food and Drug Administration (U.S. FDA) and European Medicines Agency (EMA), respectively approved Spinraza® (nusinersen), an antisense oligonucleotide (ASO) drug designed to increase the production of the SMN protein by modulating the splicing of the *SMN2* gene, thereby compensating for the underlying genetic defect. Spinraza® is administered intrathecally; an induction period is followed by life-long repeated intrathecal administrations. Despite marketing authorisation of Spinraza®, an unmet medical need for alternative treatment options of 5q SMA remains.

2.1.3. Biologic features

Spinal muscular atrophy is a motor neuron disease for which the root cause is a protein deficiency affecting a neuronal network with variable clinical thresholds (16). Motor neuron survival depends among others on a protein named survival motor neuron (SMN) protein, which is deficient in 5q SMA. Deficiency of SMN protein correlates directly with death of the individual's motor neurons. Loss of motor neurons leads to secondary effects on muscle strength and function, leading to progressive loss of muscle control, strength and function, swallowing, breathing and, ultimately, death for untreated 5q SMA Type 1.

The most common form of SMA results from bi-allelic mutations to the survival motor neuron 1 gene (*SMN1*) on chromosome 5q13 (5q SMA); of these 5q SMA cases, 95% are due to homozygous deletions, with the remainder being hemizygous deletions with a point mutation on the other chromosome. In humans, 2 forms of the *SMN* gene exist: a telomeric form (*SMN1*) and a centromeric form (survival motor neuron 2 gene [*SMN2*]). Transcription of the *SMN1* gene produces full-length messenger RNA (mRNA) transcripts that encode the SMN protein, necessary for motor neuron survival. The *SMN2* gene is nearly identical to the *SMN1* gene. A C to T substitution at position 840 results in the preferential exclusion of exon 7 from most of the transcriptional product. The resultant truncated protein is not functional. Only a small percentage (i.e., approximately 10% to 15% of the protein product of *SMN2*) is in the form of the full-length, functional SMN protein.

Individuals with SMA lack a normally functioning *SMN1* gene and are thus dependent on their *SMN2* gene. In SMA patients lacking functional SMN1, the *SMN2* copy number is inversely related to the clinical severity, with a higher number of copies associated with less severe disease. *SMN1* is ubiquitously expressed. It is unclear why motor neurons are particularly vulnerable to SMN1 deficiency.

Although the primary pathology of SMA is neurodegeneration at the level of the spinal motor neuron, some clinical reports indicate the involvement of other organs to include the heart, liver, pancreas and intestine. Metabolic deficiencies including hyperglycaemia and hypoglycaemia have been reported as well.

2.1.4. Clinical presentation and diagnosis

Several phenotypes of SMA have historically been described based on age at onset and maximal motor function achieved, although there is a consensus that these phenotypes represent a continuum from extremely severe, with disease symptoms manifesting in utero, to very mild symptoms with onset during later life. SMA type 1 presents itself with unexplained weakness or hypotonia. Molecular genetic testing with targeted mutation analysis can confirm the diagnosis of SMA.

On the basis of available newborn screening, in 2018 a treatment algorithm for infants diagnosed with 5q SMA through Newborn Screening has been published (Glascock et al., J Neuromusc Dis, 2018), however, newborn screening for 5q SMA is not an implemented standard throughout Europe yet. The algorithm recommends initiation of treatment even for pre-symptomatic 5q SMA patients that by genotype are likely to develop 5q SMA Type 1.'

The difference in severity between the SMA types is largely accounted for by *SMN2* copy number, with other genetic or environmental factors playing a minor role only. Individuals born with ≤ 3 copies of *SMN2* in the presence of a bi-allelic *SMN1* deletion or mutation have a high probability of developing a severe phenotype which, untreated, will result in significant motor function limitations including the inability to walk, high risk for respiratory complications requiring some degree of ventilatory support, high risk for orthopaedic complications such as often painful contractures and scoliosis, and truncated life expectancy.

The combination of genotypic and phenotypic characteristics led to the subdivision in SMA types presented in Table 1.

SMA Type 1 is the most common form of SMA, representing 45% to 60% of cases. The prognosis for patients with SMA Type 1 with 2 copies of *SMN2* is particularly dire; these patients show signs of the disease soon after birth (<6 months of age), never gain the ability to sit, and typically do not survive past 2 years of age without significant mechanical ventilatory and nutritional support.

Table 1: Spinal Muscular Atrophy Classification

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

SMN2 = survival motor neuron 2 gene.
bold = predominant SMN2 copy number that defines the SMA Type, the other copy numbers represent a small percentage of the designated SMA Type.
Source: Adapted from Kolb 2011 (6).

2.1.5. Management

About the product

Type of Application and aspects on development

The CHMP and CAT agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. SMA Type 1 is a severe and rapidly progressive neurodegenerative disorder, which without treatment will result in a life expectancy of less than two years. Since not all SMA subjects respond to nusinersen, there is an unmet medical need in treatment of SMA. The first results of Zolgensma with regard to survival, survival-free-of-ventilatory-support, and reaching motor milestones were considered promising. In addition, the mode of administration of Zolgensma (single intravenous infusion) and dose regime (only once) was considered a clinically relevant advantage as compared to the repeated intrathecal injections of nusinersen.

However, during assessment the CAT and CHMP concluded that it was no longer appropriate to pursue accelerated assessment, as the dossier presented deficiencies in many aspects of the Quality and Clinical presentation: With regards to the quality dossier, among others Major Objections were proposed pertaining to the following issues: control of genetic integrity of the vector, comparability of the manufacturing processes and potency assay. In relation to the clinical dossier, Major Objections were identified in relation to the amount of efficacy and safety data available with the commercial manufacturing process and the indication wording.

In addition, given the irreversible motor neuron loss in SMA, the CAT agreed to convene a SAG discuss the patient population expected to benefit most from treatment with Zolgensma.

Therefore, the evaluation reverted to standard timetable.

The applicant also requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14-a of the above-mentioned Regulation, based on the following criteria:

The benefit-risk balance is positive and Zolgensma is designed to address the monogenic root cause of SMA by replacing the defective *SMN1* gene resulting in increased levels of SMN protein.

- It is likely that the applicant will be able to provide comprehensive data:

Data from Studies CL-303, CL-302 and CL-304 are anticipated to address the uncertainties of an initial CMA based on preliminary data from the ongoing phase III studies and on CL-101. Pivotal Study CL-303 has a combined primary efficacy endpoint, has been completed as of 31 DEC 2019 and the final Clinical Study Report is expected to be available for submission in September 2020. In addition, the quality of data generated in Study CL-303 and the remaining studies in the Zolgensma clinical development program (including Study CL-302 and CL-304) will not be affected by approval of its CMA as all studies are open-label, single-arm trials and, therefore, study blinding (i.e. none) and statistical analyses will not be compromised.

- Unmet medical needs will be addressed:

Although another available treatment option is nusinersen, nusinersen treatment is associated with significant burden for the patient since it requires lifelong intrathecal injection, which is associated with safety risks. Zolgensma effectively targets the disease mechanism in 5q SMA, has improved efficacy and a more convenient method of administration when compared to those of nusinersen; and has at least a comparable safety profile to that of nusinersen.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

Whilst the confirmatory Study CL-303 is completed as of 31 DEC 2019, the final CSR is awaited. Early access to this innovative treatment option would be highly important for especially SMA Type 1 patients, which without treatment will result in a life expectancy of less than two years. In 2017, nusinersen was authorised as a disease-modifying treatment resulting in advances in SMA patient care and outcomes. However, not all treated patients have improved their muscular functions as demonstrated by the ability to sit or walk unassisted.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as solution for infusion containing 2.0 x 10e13 vg/mL of onasemnogene abeparvovec as active substance.

Other ingredients are: tromethamine, magnesium chloride, sodium chloride, and poloxamer 188, hydrochloric acid (for pH adjustment) and water for injection.

The product is available in 10 mL crystal zenith vials with a nominal fill volume of 5.5 mL or 8.3 mL.

2.2.2. Active Substance

General Information

Zolgensma is a single-dose, preservative-free, sterile, clear to slightly opaque, and colourless to faint white, intravenous infusion of non-replicating, self-complementary AAV9 vector. Zolgensma contains the human survival motor neuron gene (*SMN1*) under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB). One of the two adeno-associated vector (AAV) inverted terminal repeats (ITRs) has been modified to promote intramolecular annealing of the transgene, thus forming a double-stranded transgene ready for transcription. The size of the packaged self-complementary vector genome is ~4.6 kb.

The capsid is comprised of 60 viral proteins (VP1, VP2, VP3) in a ratio of approximately 1:1:10 that are derived from the AAV serotype 9. The capsid proteins are produced by alternate splicing such that VP2 and VP3 are two truncated forms of VP1, all with common C-terminal sequences. The predicted molecular weights of VP1, VP2 and VP3 are 81.4, 66.3 and 59.8 kDa respectively.

The onasemnogene abeparvovec AS (active substance) is a clear, to slightly opaque, colourless to faint white solution at a concentration of 3.4 to 7.3 x 10e13 vector genome-containing particles per millilitre.

Manufacture, process controls and characterisation

Manufacturing facilities

The manufacture of onasemnogene abeparvovec takes place at AveXis, Inc., Libertyville, IL 60048, USA. The site has been inspected by HPRA and is covered by a GMP certificate.

Description of the manufacturing process and process controls

The manufacturing process of Zolgensma AS is a complex process involving several steps in upstream and downstream manufacturing, whereby the maximum duration of all steps leads to an overall process time of several days.

The AS Upstream manufacturing process consists of five steps, or unit operations: 1) Cell Expansion, 2) Bioreactor Operations, 3) Bioreactor Harvest, 4) Harvest Clarification, and 5) Intermediate.

During Upstream manufacturing, one vial of the human embryonic kidney cells (HEK293) working cell bank (WCB) is thawed, and cells are expanded. The expanded cells are harvested and used to inoculate the bioreactor. The cells expanded are transfected with a triple DNA plasmid solution. After cell culture, the cells are harvested and clarified. The clarified harvest is processed to Intermediate and frozen. All open cell/product manipulations in the Upstream manufacturing process occur inside a biosafety cabinet (BSC) within an ISO 7 area. Media, solutions, and buffers that are prepared by AveXis are prepared in an ISO 8 area.

The AS Downstream manufacturing process includes thawing and pooling of the intermediate, clarification process, chromatographic purification step, filtration and centrifugation steps and filling of the AS. No reprocessing is allowed.

Overall, the manufacturing process is well described including detailed flow charts. In-process controls are in line with the expectations.

Control of Source and Starting Materials of Biological Origin

The Starting Materials for onasemnogene abeparvovec consist of a mammalian cell bank and three recombinant plasmids. An overview of raw materials used in the manufacturing of the active substance has been provided including information on their intended use, whether they are compendial or non-compendial, and specifications for non-compendial material. Information on the vendors of critical raw materials is provided. Specifications and representative certificates of analysis are provided.

For the starting materials, information on the source, history, and generation of the plasmids and the cell banks has been provided. The applicant used a vial of HEK 293 cells to create a pre-MCB (master cell bank). Subsequently a MCB and three WCBs (working cell banks) were manufactured under GMP. All three generated WCBs will be used for manufacture of clinical and commercial material.

Zolgensma AS is produced by co-transfection of HEK293 cells withthree plasmids:
• Vector Plasmid (pSMN)
• AAV Plasmid (pAAV2/9) containing the AAV rep2 and cap9 wild-type genes
• Adenovirus Helper Plasmid (pHELP).
Plasmid DNA maps are provided. The plasmid manufacturing and testing sites are provided.

The tests and/or acceptance criteria for the plasmid DNA batches manufactured by the two plasmid production sites show some differences, which have been sufficiently justified. An overview was provided of the plasmid DNA batches (manufacturer and batch number) that have been used for the production of the different commercial AS batches listed in the MAA.

Control of critical steps and intermediates

Tabular overviews of the critical and key process parameters (CPPs and KPPs), in process controls (IPCs) and in process acceptance criteria (IPACs), including their respective ranges, action limits, and acceptance criteria are provided.

Relative wide operating ranges are defined for some of the key process parameters and IPCs; the applicant committed to periodically evaluate key and critical operating ranges as well as action limits.

The selection of in process tests is acceptable.

Validation

The Applicant completed consecutive process verification runs at commercial scale for each of the three manufacturing stages (cell expansion, intermediates and downstream manufacturing). A total of five runs were executed under the vial thaw and cell expansion protocol. Five runs through Intermediate were executed using a small-scale bioreactor, and three runs were executed using the commercial scale

bioreactor. Four process verification batches were executed for the downstream process. Overall, the pre-defined acceptance criteria were met for most of the process parameters with only few exceptions, which had no impact on forward processing.

Development

Research preparations of Zolgensma were used for non-clinical studies and/or proof of concept studies. Details regarding manufacturing and release testing are not provided. As it is claimed that these non-clinical studies were repeated with material from Process B the absence of information regarding research preparations is accepted.

Two different processes, A and B, have been used to manufacture vectors for clinical evaluation. One batch is manufactured at a research institute, called Process A. Only this Process A batch is used in the Phase 1 clinical study that supports the present application.

Subsequent batches are referred to as Process B batches and claimed to be manufactured with the commercial process. However, the upstream manufacturing of the first two Process B AS lots was performed in 2017 at a different site prior to transferring the upstream process to AveXis were also the downstream Process B takes place. The manufacturing site of AveXis at Libertyville, IL, USA is the commercial manufacturing site of Zolgensma.

Overall the changes between upstream process A and process B are sufficiently described and related to the change of production sites, upscaling and introduction of bioreactor, change of equipment, as well as process optimisation and improvement of robustness and consistency. The changes for the downstream Process B were extensive and Process A and Process B are not comparable as such. In addition a MCB/WCB of HEK293 cells was introduced for Process B and the slightly modified plasmids were manufactured by a commercial supplier. Due to changes during development and with the data provided, it cannot be demonstrated that the Process A batch at the time of its manufacturing / clinical use is comparable to the Process B batches. The assessment of the benefit risk of Zolgensma therefore needs to be based on the clinical data obtained with Process B batches.

Comparability of the Process B batches is discussed in the Finished Product section below.

Characterisation

Elucidation of structure and other characteristics
The active substance has been sufficiently characterised by physicochemical and biological state-of-the-art methods revealing that the active substance has the expected structure. The analytical results are consistent with the proposed structure.

The characterisation study commences with a purification by gradient centrifugation of one Process B FP batch to produce a band profile. Each band of proposed full capsid material was isolated and further characterized by SDS-PAGE analysis, genomic (RNA/DNA) isolation, *in vitro* SMN expression analysis, residual DNA analysis, genomic titre by PCR, total protein content, analytical ultracentrifugation (AUC) and sequencing.

The analysis of the capsid proteins suggests slight differences in residual DNA levels and capsid protein ratio among the bands. The isolated bands are shown to be capable of transducing cells and expressing the encoded SMN gene indicating the presence of functional and infectious particles.

It remains to be demonstrated whether ratio of the bands and capsid protein ratio are comparable among batches and how the ratio is affected by the production process. The applicant committed to performing additional studies to characterize bands.

The results from the forced degradation study demonstrate the sensitivity of Zolgensma to acid hydrolysis, base hydrolysis, and oxidation. Zolgensma is not sensitive to light.

Impurities
The concentrations of process-related impurities were measured at various stages of the manufacturing process to evaluate the level of removal. Process-related impurities were shown to be reduced to acceptable concentrations.

Data on product-related impurities has been provided and is based on forced degradation studies summarised in the stability section. Empty capsids and rcAAV are controlled during AS release and thus no additional characterisation data are required for these impurities.

Upon request, further information was provided on the control of aggregates. Preliminary data support that Zolgensma may not be prone to aggregate formation and aggregates could be detected by SV-AUC. Further evaluation, however, showed that the latter method is not adequate for monitoring aggregates. The applicant committed to further develop quantitative aggregation assay and include this test in the stability test panel once validated.

The applicant committed to determine the amount of co-packaged cap, rep, host cell DNA and adenoviral sequences.

Specification, analytical procedures, reference standards, batch analysis, and container closure

Specifications
Release test results from 20 consecutive Zolgensma AS lots manufactured by the proposed commercial process were evaluated to develop the proposed acceptance criteria. The data demonstrates the consistency of the AS lot yield as measured by the total number of vector produced.
The AS specifications are defined based on manufacturing and clinical experience, and pharmacopoeia standards. Active substance is tested for pH, appearance, osmolality, titre, identity, impurities, and microbiological attributes.

Overall the specifications of the AS are sufficiently justified. A commitment is provided to develop a more sensitive and precise method for analysis of protein impurities and re-evaluate the specifications for impurities based on the sensitivity of the new method.

Analytical procedures
Summaries of the analytical method principles, detailed SOPs and validation reports are provided. All methods have been validated in line with ICH Q2.

Reference standard
Due to the similarity in formulation of AS and FP, the FP reference material can be deployed for testing of AS as well as FP. Reference standards are further described with the FP.

Container closure system
The AS is stored in sterile bottles. Bottles are filled and frozen. Sufficient information on the AS container closure system is provided, including specifications and technical drawings. The extractables and leachables studies support the use of bottles for the storage of Zolgensma AS.

Stability

The AS stability study comprises tests for Appearance, pH, Osmolality, Genomic Titre, Purity and Total Impurities. Stability acceptance criteria are the same as release criteria. Small containers representative of the actual AS containers were used for storage. Long-term primary stability studies at the long-term storage condition are ongoing for the active substance process B lots manufactured at Avexis.

In addition, the applicant committed to develop methods to detect aggregates and improve detection of degradation products.

2.2.1. Finished Medicinal Product

Description of the product and Pharmaceutical Development

Zolgensma finished product is a single-dose, preservative-free, sterile, clear to slightly opaque, and colourless to faint white, intravenous infusion of non-replicating, self-complementary AAV9 vector at a target concentration of 2.0 x 10^{13} vg/mL.

Zolgensma FP solution in Water for Injection (WFI) contains Tromethamine (Tris), Magnesium Chloride, Sodium Chloride, and Poloxamer 188. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. Zolgensma FP is filled into 10 mL crystal zenith vials with a nominal fill volume of 5.5 mL or 8.3 mL and stored at ≤ -60°C. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Pharmaceutical development

Zolgensma AS contains the same buffer excipient matrix that is used for the concentration adjustment of Zolgensma FP. The justification for the formulation of Zolgensma is sufficient. The dossier contains a detailed justification for the selection of the excipients and their functionality.

Physicochemical and biological properties

Information is provided on the vector quantity, finished product density, potency and its control.

Manufacturing process development

Process changes

Three finished product manufacturing processes are described: Process A (manufactured at a research institute), Process B-initial, and Process B-commercial (both manufactured at Avexis). Only one clinical batch manufactured according to Process A is listed, which was used in the Phase I clinical study that supports the present application; the batches manufactured according to Process B–initial were used in development and in the ongoing pivotal clinical trial. Batches manufactured according to Process B-commercial were used for process validation and used in clinical studies.

The finished product presentation is different for each process with regard to container closure system, fill volume, and vector concentration. These changes were implemented to support long-term storage and achieve the desired dose and dose volumes. With regard to the finished product manufacturing process, the main difference is the presence of an active substance thaw step in Process A and Process B–commercial, which is not present in Process B–initial. Manual filling in Process A was replaced by an automated filler in Process B-initial and -commercial.

Comparability

As discussed in the section on Active Substance, no conclusions can be drawn on comparability between the Process A batch and Process B batches. The assessment of the benefit risk of Zolgensma therefore needs to be based on the clinical data obtained with Process B batches.

At D150, no comparability assessment of Finished Product manufactured according to Process B-initial and Process B-commercial had been performed It was noted that the available batch analysis data of Process B batches showed no obvious differences, to Process A batches. However, the infectious titre per 10^{13} vg showed relatively large batch-to-batch variation, and comparison of the data was hampered by changes that were made to the test methods and not justified by data demonstrating that the revised

methods yielded equivalent results. Also, stability data for the Process A batch suggested that a shift in the measured titre may have occurred during development due to a change in method.

As accurate dosing of Zolgensma is critical, additional data were requested to address above uncertainties and demonstrate assurance of comparability of the Process B-batches with regard to vector titre and infectivity.

The response of the applicant showed that only a Phase I clinical batch is impacted by the shift in the measured titre due to a change in the analytical method. All Phase 3 clinical batches and PPQ/commercial batches were tested using the revised test method and show consistent genomic titre. Also, additional data are provided to demonstrate that the batch-to-batch variation in infectious titre calculated per 10^{13} vg can be attributed to assay variability. Infectivity of the batches may, however, also be deduced from the *in vitro* relative potency assay, that measures protein expression relative to a finished product reference batch (see below). Finally, preliminary data on the impact of freeze-thaw are provided. No impact on product quality is observed, however, further studies, including additional batches and also evaluating potential aggregation, should be performed to confirm this result. A commitment was provided by the applicant to perform these studies.

The applicant demonstrates that the relative protein expression measured in the *in vitro* potency assay closely correlates with the relative infectivity (percentage positive cells) in the *in vitro* potency assay. It is therefore in principle agreed that the *in vitro* relative potency assay is used to control lot-to-lot consistency of the effective dose (i.e. the amount of administered particles that are capable of transducing cells). However, careful monitoring of the reference standard stability and its performance is required to ensure adequate and consistent potency control. *Control Strategy*

A risk-based approach was adopted for the assignment of CQA's. In general, the identified CQAs are the parameters tested for active substance and finished product release. This can be accepted.

A process parameter risk assessment was performed via Failure Mode and Effects Analysis (FMEA). The approach used to determine criticality of process parameters can in general be accepted. Operational range for critical (CPPs) and key (KPPs) process parameters are sufficiently justified.

Processing/hold durations can be accepted as they are either below 24 hours or supported by process validation data. The duration of sterile filtration and filling are identified as CPP.

Container Closure System

Sufficient information on the selection of the container closure system is provided. The selected container closure system is expected to be suitable for its intended use. Characterisation studies confirmed container closure integrity. Compatibility of the finished product with the container closure system (extractables and leachable testing) and of the finished product with a syringe and infusion set that are needed for administration to the patient was evaluated. The provided information gives no reason for concern. Overall, storage in the respective syringe for 8 h and infusion duration of 60 min is supported.

Manufacture of the product and process controls

Manufacturers

Manufacturers, contract testing laboratories and contract storage sites for the Finished Product are listed in table 6 below. GMP compliance for Avexis Inc. and contract laboratories involved in manufacture, testing and storage of Zolgensma was confirmed. Import of the FP, secondary packaging, EU batch certification, and storage and distribution is performed by Almac Pharma Services Ltd., Ireland.

The Applicant has provided a justification, in accordance with paragraph 11.17 of Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products (Eudralex The Rules Governing

Medicinal Products in the European Union, Volume 4, Good Manufacturing Practice), to rely on the finished product release testing performed in the US, claiming a limited amount of material available. The CAT agreed to the justification given by the applicant that in view of the current small batch size which is limited due to the batch allocation strategy, re-testing in the EU would consume a disproportionate amount of this batch size. Therefore, the current testing plan has been accepted for this specific small batch size. Should the applicant consider a batch upscaling or registration of new sites in the future, omission of the batch release re-testing in the EU will need to be re-considered.

Description of the manufacturing process and process controls

The description of the manufacturing process is considered adequate. A detailed description of the manufacturing process is provided, including a flow diagram and narrative of how each step is performed. Briefly, the finished product manufacturing process consists of active substance thawing and pooling, sterile filtration and concentration adjustment, filling, visual inspection, labelling and secondary packaging. Tabular overviews of the critical and key process parameters (CPPs and KPPs), in process controls (IPCs) and in process acceptance criteria (IPACs), including their respective ranges, action limits, and acceptance criteria are provided. The selection of in process tests is acceptable. All unit operations in the finished product manufacturing process are identified as critical.

During the filtration step, the active substance is diluted to reach the target concentration of 2.0×10^{13} vg/mL (IPC).

No intermediates are described in the finished product manufacturing process. In process-hold times are supported by process validation.

Process validation and evaluation

The Applicant completed four consecutive PPQ runs. The manufacturing process was performed at set point conditions for the critical and key process parameters.

Two out of four process verification runs were successfully completed (run 3 and 4). For these batches, all acceptance criteria for IPCs and Finished product release were met. In process verification run 2, the sterile filtration had to be repeated due to a system leak prior to filling. Reprocessing of the sterile filtration step is validated (see above); the release specifications were met in PPQ run 2. In PPQ run 1, the acceptance criterion for titre after the sterile filtration/concentration adjustment step (IPC) was not met. An additional filtration step and sterile filtration were performed to correct the titre. This reprocessing option had also been included in the manufacturing process description but was deleted upon request.

Overall, the provided validation data show that, when operating at set-point conditions, the manufacturing process is capable to consistently yield a product meeting its predefined acceptance criteria. Upon request, additional data were provided to further support the proposed operational ranges and manufacturing scale.

The information provided on the additional validation studies (media fill studies, filter validation, shipping validation) in general give no reason for concern.

Product specification, analytical procedures, batch analysis

Finished product specifications

Finished product is tested for pH, appearance, osmolality, titre, impurities, potency, particulates and microbiological attributes.

The proposed test panel for the finished product is acceptable, taking into account the commitment of the applicant.

Analytical procedures

Detailed descriptions of the analytical procedures deduced from the respective SOPs have been provided. The analytical procedures were adequately validated.

Batch analysis

Batch analysis data are provided, including data from Process A, Process B-initial batches, the PPQ batches (Process B-commercial), and Process B-commercial batches.

Justification of specifications

The Applicant has provided a detailed justification of the proposed acceptance criteria for each of the tests performed for finished product release. For quantitative criteria, a statistical analysis was performed and limits were set.

In response to the request to determine whether tightening of the FP release specifications limits is required to ensure optimal clinical outcome (Major Objection), the applicant has evaluated batch release data and the relation between clinical outcome and FP attributes. It is agreed that this evaluation did not identify a need to tighten the specifications. However, clinical data are considered currently too limited to reach a final conclusion. The applicant committed to perform a further evaluation of the FP specifications when additional patient data are available.

Due to the concerns with regard to stability of the reference standard, the applicant was asked to demonstrate that the acceptance criterion for potency provides a suitable and consistent target potency (Major Objection). This issue is not fully solved. However, consistency of the potency measurement is further ensured by the committed implementation of an assay control and adequate monitoring of the reference standard stability and performance. In addition, as discussed above, the applicant will perform further evaluation of the acceptance criteria when additional patient data are available.

Reference standard

The finished product lot manufactured according to Process A was used as interim reference standard. Qualification was based on the release test panel that was in place at the time of manufacture.

The primary reference standard is qualified by release testing and additional testing. As part of the qualification, bridging with the interim reference standard was performed. The questions raised regarding the bridging approach were not solved but will not be pursued.

The general approach that will be used for preparation and qualification of additional primary and working reference standards is acceptable. The Applicant acknowledged that, in the absence of an approved protocol for the qualification and bridging of future reference standards, a variation application should be submitted for implementation of new reference standards. Stability of the reference standards will be confirmed by assay monitoring and requalification using a subset of release, stability and characterisation test methods.

Container closure system

The primary container closure system for Zolgensma FP is a clear 10 mL crystal zenith vial with a chlorobutyl elastomeric stopper, and an aluminium seal with a coloured plastic button cap. Sufficient information on the container closure system is provided, including specifications and technical drawing, and information on extractables/ leachables and microbiological attributes. Container closure integrity is confirmed.

Stability of the product

Stability studies have been performed in accordance with current ICH/CHMP guidelines.

The stability test panel includes general tests (appearance, pH, osmolality), potency-related tests, purity, and container closure integrity. Upon request, a test for sub-visible particles was added to the long-term stability protocol. An aggregation assay is under development and will be added to the stability program once validated.

The available long-term stability data include 12-month stability data for PPQ, supportive, and commercial FP lots stored at <-60°C. All acceptance criteria are met, however, some downward trends have been observed. The applicant committed to continuously monitor genomic titre change during stability and evaluate the potential root cause.

The shelf-life for Zolgensma FP at the long-term storage condition of < -60°C is 12 months. This can be accepted, but the shelf-life should be re-evaluated when additional stability data are available, including data on aggregates and the outcome of the root-cause analysis. All batches are also included in accelerated (2-8°C) and stressed (20-25°C) stability studies.

The Zolgensma FP post-approval stability commitment is to continue the ongoing stability programs as described in Section 3.2.P.8.1 in support of the proposed shelf-life. Additionally, at least one Zolgensma FP commercial lot at each fill volume will be placed into the stability program annually at the long–term storage condition of ≤ -60°C. The stability data on which the summary and conclusion in P.8.1 is based, are included in the dossier.

As stated in the SmPC the product should be stored in a refrigerator (2 -8°C) immediately upon receipt. Once thawed, the medicinal product should not be re-frozen and may be stored refrigerated at 2°C to 8°C in the original carton for 14 days. Once the dose volume is drawn into the syringe it must be infused within 8 hours. Discard the vector containing syringe if not infused within the 8-hour timeframe.

Post approval change management protocol(s)

N/A

Adventitious agents

The Applicant has provided a general overview of the adventitious agents safety evaluation, including information on the control of materials, the testing for potential adventitious agents, and viral clearance studies.

Non-viral adventitious agents

Control of contamination of the manufacturing facility (e.g. environmental monitoring, cleaning procedures) are briefly described in section 3.2.A.1; Media fill studies are described in section 3.2.P.3. and have been successfully performed.

With regard to the testing performed for non-viral adventitious agents, for cell bank testing, and the Active substance manufacturing process reference is made to CTD section 3.2.S.2.3. The cell banks tested negative for bacteria, fungi and mycoplasma. In process controls for bioburden and endotoxin are in place. The Active substance release tests include bioburden; Finished product is tested for sterility and endotoxin. The provided information suggests adequate control of non-viral adventitious agents.

Compliance with the "Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" (EMA/410/01 rev 03) has been

sufficiently shown for all reagents and equipment used at Zolgensma manufacture and cell bank generation.

Viral adventitious agents

Cell bank testing for viruses is described in CTD section 3.2.S.2.3. The commercial MCB is tested in line with ICH Q5A, including NAT test panels for human viruses and AAV, and *in vitro* assays for bovine and porcine viruses. WCBs are tested for viral contaminants *in vitro* using 3 cell lines.

All cell banks were found negative for all viruses tested except for a low signal with the PERT assay. Even though such result is not unexpected for this highly sensitive assay, the applicant was asked to clarify if an infectivity assay has been performed as required by Ph. Eur. 5.2.3 under such conditions. In response, the applicant justified the retrovirus testing scheme and committed to perform an infectivity assay for the next MCB in case of positive Q-PERT assay. Upon request, the applicant also provided data on virus testing on end-of-production (EOP) cells derived from the current three WCBs.

Production control cells, pre-lysed harvest and the intermediate are tested for viral *contaminants in vitro*, which is in line with Ph. Eur. and ICH Q5A. The Active Substance release tests include a test for rcAAV. A discussion of the risk of replication competent AAV is included in section 3.2.A.

Animal and human derived material

The listed animal derived materials used in the cell bank generation and cell culture are serum, cell disassociation agent, and production media containing human transferrin.

The serum used is sourced from animals in New Zealand and gamma irradiated. A certificate of suitability was provided for the serum used at MCB and WCB manufacture and used for production.

Production medium used as cell culture medium after transfection contains human transferrin. The source plasma is collected from U.S. donors in FDA approved centres, thus the TSE risk is deemed negligible. The information provided for human transferrin with regard to plasma testing (HIV 1/2 Ab, HCV Ab, HbsAg; NAT testing for HAV, HBV, HCV, HIV-1 and PB19) and virus inactivation procedures (heat treatment of the product for 10 hours at 60°C) is noted. Upon request, only transferrin for which information on quality and control is provided in line with Chapter 10 of the Guideline on plasma-derived medicinal products (EMA/CHMP/BWP/ 706271/2010) will be used in Zolgensma manufacture.

Virus validation studies

Virus inactivation and clearance studies were performed. All three steps were validated for virus inactivation / removal in down scaled spiking studies. X-MuLV, PRV, HAV, and MVM were used as model viruses whose choice is acceptable. The down scaling was adequate.

The results of the virus validation studies suggest that the manufacturing process is capable to effectively inactivate/remove enveloped viruses (log reduction > 8).

GMO

See section 2.3.4.

2.2.2. Discussion on chemical, pharmaceutical and biological aspects

The manufacturing process of Zolgensma is sufficiently described and has been adequately validated at set point conditions.

Sufficient information is provided on the manufacturing of the plasmids and the generation of the HEK-293 MCB and WCBs that are used for the manufacturing of the vector. Testing of the cell banks complies with requirements. Control of the plasmid is performed by the plasmid manufacturers.

Three manufacturing processes are described during development: Process A, Process B-initial, and Process B-commercial. Major changes are made between Process A and Process B. Comparability of batches manufactured according to Process A and batches manufactured according to Process B has not been demonstrated. However, as the single Process A batch is > 4 years old and bridging of the test results is not possible this issue (D120 Major Objection) was not further pursued. As comparability of the Process A batch and Process B batches cannot be demonstrated, the assessment of the benefit risk of Zolgensma will be based on clinical data obtained with Process B batches.

The main differences between Process B-initial and B-commercial include the container closure system, fill volume, and vector concentration and the presence of an Active Substance freeze-thaw step in Process B–commercial, which is not present in Process B–initial. For Active Substance, all Process B batches are claimed to be manufactured according to the commercial process.

As the therapeutic window of Zolgensma appears to be narrow, accurate dosing is critical. It is therefore important that 1) consistency of the Process B batches with regard to e.g. vector titre and infectivity is demonstrated and 2) an impact of the change from Process B-initial to Process B-commercial is excluded. Preliminary data suggest that the additional Active Substance freeze-thaw step does not impact product quality. The applicant committed to perform additional studies to confirm this. In addition, the applicant committed to re-evaluate the finished product specifications when additional patient data are available, in order to determine whether the release specifications adequately ensure optimal clinical outcome.

The characterisation study and its evaluation are limited. It remains to be demonstrated whether ratio of gradient centrifugation bands and ratio of capsid proteins is comparable among batches and how the ratio is affected by the production process. The applicant provided a commitment to perform additional studies. The strategy of pooling the bands is justified as pooling has been used in non-clinical as well as in clinical application, resulting in the current safety and efficacy profile. However, the differences between the vector particles from the bands is not fully understood. Thus, the safety and/or efficacy profile of the product may benefit from evaluating the relevance of using all bands in manufacture of the product.

The Active Substance and Finished Product specifications can be accepted with the commitment to implement a qualified genomic integrity assay. As discussed above FP acceptance criteria will be re-evaluated when additional patient data are available. The proposed shelf-life for Zolgensma FP (12 months at < -60°C) is accepted but should be re-evaluated when additional stability data are available. In-use stability data have been provided and give no reason for concern however, studies are not completed yet. The applicant committed to provide post-thaw stability data at the end of Finished Product shelf life and data on potential aggregate formation to further support the claimed in-use storage period. The SmPC contains a statement that, once thawed, the product should not be re-frozen, and may be stored for 14 days at 2-8°C.

2.2.3. Conclusions on the chemical, pharmaceutical and biological aspects

The CAT has identified the following measures necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product:

The applicant should perform a further evaluation of the finished product specifications when primary and key secondary endpoint data from additional patients with 2 copies of SMN2 are available (i.e. completion of CL-302 and CL-304 cohort 1). Based on this evaluation, it should be determined whether

tightening of the release specification limits is needed to improve consistency of the batches and ensure optimal clinical outcome.

The evaluation should take into account 1) product stability and 2) the correlation between critical quality attributes and clinical outcome in terms of survival, ventilatory support, motor milestones and relevant motor function scores (Bayley) in (pre-)symptomatic subjects born with 2 SMN2 copies.

The correlation evaluation should include a complete evaluation of the range of FP data against clinical data (e.g. comparing the higher and lower ranges of critical quality attributes) and consider whether batches at the end of shelf life perform as well as batches at the beginning of shelf life. The analysis will take into account known sources of variability in assays and measurement techniques.

The estimated dose given to the patient (vg/kg as determined based on the estimated genomic titre of the product batch at the time of dosing taking into account stability data) should be included as additional quality attribute.

The CHMP endorsed the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

2.2.4. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommends the following points for investigation:

1. Control of vector genome integrity: The applicant should develop and implement a release test and define scientifically justified acceptance criteria. When results of the first 30 batches tested with the in-house method are available, the continued need for a release test for vector genome integrity may be re-evaluated.

2. The applicant will submit a variation to expand the release testing panel in the EU in 2022, before the implementation of additional manufacturing capacity.

3. The applicant commits that at least four lots of intermediate will be included into the CPV program. Analysis of the intermediate from the first CPV will be provided with the first renewal of the conditional marketing authorisation application.

4. The applicant will develop a more sensitive and precise method for analysis of protein impurity by end of 2020 and will submit a variation in 2021. Additionally, the specifications of impurities will be assessed in conjunction with the development of this method and modified as appropriate.

5. The applicant will perform LC-MS analysis in order to attempt to identify the impurities listed in the specification. A progress update will be provided by June 2020.

6. The applicant will validate an aggregation assay and, once validated, submit a variation to add this method to the stability program for finished product in 2021.

7. The applicant commits to further characterize the aggregation for multiple batches ensuring that end of shelf-life samples are included in the characterisation. Data will be provided with the first application for renewal of the conditional marketing authorisation application.

8. The applicant commits to revalidate the sterility test method to demonstrate absence of interference for Ph. Eur. compliant sampling volumes by June 2020.

9. The applicant commits to perform a study to further confirm the *in vitro* and in vivo correlation using additional 2 finished product lots at the optimal dose level identified in the first phase of the study. The data should be submitted once available.

10. The applicant commits to establish an independent assay control for the potency assay by August 2020 to monitor assay performance as well as reference standard stability.

11. The applicant commits to evaluate the impact of freeze thaw on active substance potency in at least two other active substance batches. The selection of batches/samples should aim to cover the range of active substance strength. Evaluation of the impact on aggregation should be performed once a suitable method is available. At first conditional authorisation renewal.

12. The applicant commits to perform additional studies to characterize gradient centrifugation bands and demonstrate lot-to-lot consistency and comparability of batches manufactured according to Process B-initial and Process B-commercial at first conditional authorisation renewal.

13. The applicant commits to determine the amount of co-packaged cap, rep, host cell DNA and adenoviral sequences and provide the results with the first renewal of the conditional marketing authorisation.

14. The applicant commits to investigate possible root causes of the observed downward trends during stability. In addition, potency data will be collected for selected samples from these studies to evaluate potency change during storage. If such decrease is confirmed, additional development will be performed to evaluate factors that can impact stability during frozen storage. Progress update at first conditional authorisation renewal.

15. The applicant commits to re-evaluate the finished product shelf life when further stability data are available and to include 1) data on aggregates and 2) the results of the root cause investigation. Progress update at first conditional authorisation renewal.

16. The applicant commits to provide end of shelf life post-thaw storage and performance stability data and data on aggregates with the first renewal of the conditional marketing authorisation at first conditional authorisation renewal.

17. The applicant will perform shock and vibration studies and additional shipping validation studies for the load of vials prior to the first commercial shipments of Zolgensma in the EU.

18. Any unexpected genomic integrity results (e.g. results indicating mutations in the expressions cassette of the therapeutic gene which may lead to non-functional protein or altered protein levels) should be reported immediately.

The CHMP endorsed the CAT assessment regarding the recommendation(s) for future quality development as described above.

2.3. Non-clinical aspects

2.3.1. Pharmacology

Overall, the pharmacology studies in a knockout model in mice provide proof of principle for the clinical treatment rationale for IV injection of Zolgensma in the intended patient population (young patients with SMA type 1). IT injection of Zolgensma in an induced model of SMA1 in piglets provide additional proof for the concept. IV injection with AVXS101 at early time points (PND 1 or 2) in mice or NHP and IT and ICV injection in juvenile piglets, show efficient systemic and CNS biodistribution of AVXS101 as well as efficient transgene expression. In addition, pharmacology studies in mice and cynomolgus monkey suggest that early treatment is beneficial, which was also observed in rat and cat according to published data. The later treatment is started, the more motor neurons are damaged, which cannot be cured by this therapy. Furthermore, the later treatment is started in mice, the more glial cells and the lesser motor neurons are transduced, although transduction of motor neurons is key to treatment success.

Intrathecal (IT), intracerebroventricular (ICV) or intracranial injection shows efficient distribution of the vector to the motor neurons in the CNS of NHP using a 10-fold lower dose compared to IV injection. ICV dosing in mice largely limited biodistribution to the CNS whereas upon IT injection in monkeys also peripheral tissues were efficiently transduced, suggesting that the monkey is also exposed systemically upon IT dosing. However, the IT route is not the administration route intended in the proposed indication and thus, studies using this administration route were only considered as supportive studies.

Knockout mouse model for SMA; smnΔ7 mice; IV and ICV dosing

Distribution of AAV9 and subsequent transgene expression was assessed in neonatal and adult mice. Targeting of AAV9.CB.GFP, a AAV9 vector similar to Zolgensma but containing a sequence for GFP protein, was studied in wild-type C57BL/6 mice that received an IV injection of AAV9.CB.GFP on PND1 or 2, when the blood-brain barrier in mice is not fully developed. GFP expression at 10 and 21 days post injection was identified in heart and skeletal muscle, in dorsal root ganglia (DRG) and lower motor neurons (LMN) in the spinal cord. Widespread GFP expression was found in the brain, which also persisted up to 21 days. In contrast, IV injection in adult mice (~70 days) did not result in GFP expression in DRG and markedly decreased LMN transduction. GFP expression in spinal cord of animals injected at adult age was identified primarily in astrocytes. In the brains, GFP expression was restricted primarily to dentate gyrus and hippocampus, suggesting a markedly different expression pattern in adults compared to neonates. GFP expression was maintained for up to 7 weeks post induction.

In the pharmacology study conducted by Foust et al., 2010, timing of administration of AAV9-SMN1 was assessed in a mouse SMA model (smnΔ7 mouse). Mice received AAV9-SMN1 at PND 1, 2, 5 or 10. Survival and motor function of AAV9-SMN1 injected animals on PND2 appeared similar to injection on PND1. In addition, size and weight was improved compared to the placebo smnΔ7 group, but not reach the levels of control mice (*SMN2*$^{+/+}$; SMNΔ7$^{+/+}$; *Smn*$^{+/-}$). Mice given AAV9-SMN1 on PND5 showed a shift from neuronal to glial transgene expression, suggesting a change has occurred in blood-brain barrier development. Survival and motor function were modest, with no animals surviving beyond approximately 30 days. Injection of AAV9-SMN on PND10 resulted in no rescue of phenotype and survival was comparable to control GFP-injected smnΔ7 mice. This suggests that timing of administration is important to obtain an optimal clinical effect. In addition, treatment at PND10 may be too late as the accumulated neuronal damage in the first 10 days of life is sufficient to initiate early death. Rather, administration of 3.3 x 10^{14} vg/kg AAV9-SMN on or before PND2 is able to drastically increase survival and motor function in a mouse model of SMA. In the written summary only, dose selection results are presented, but are not supported with publications or underlying data (see figure 2.1.2.1.b in NC AR). This work was not part of the publication of Foust et al. 2010. It appears that, based on this figure alone, at the highest dose tested (3.3 x 10^{14} vg/kg), mice survived for over 250 days and the dose immediately below that (6.7 x 10^{13} vg/kg) is no longer effective. The MTD in the pivotal toxicology studies is 1.5 x 10^{14} vg/kg, which suggests absence of a therapeutic window. In the clinical part of the dossier a similar figure (assessor's comment figure 1) was shown as compared to the figure discussed here.

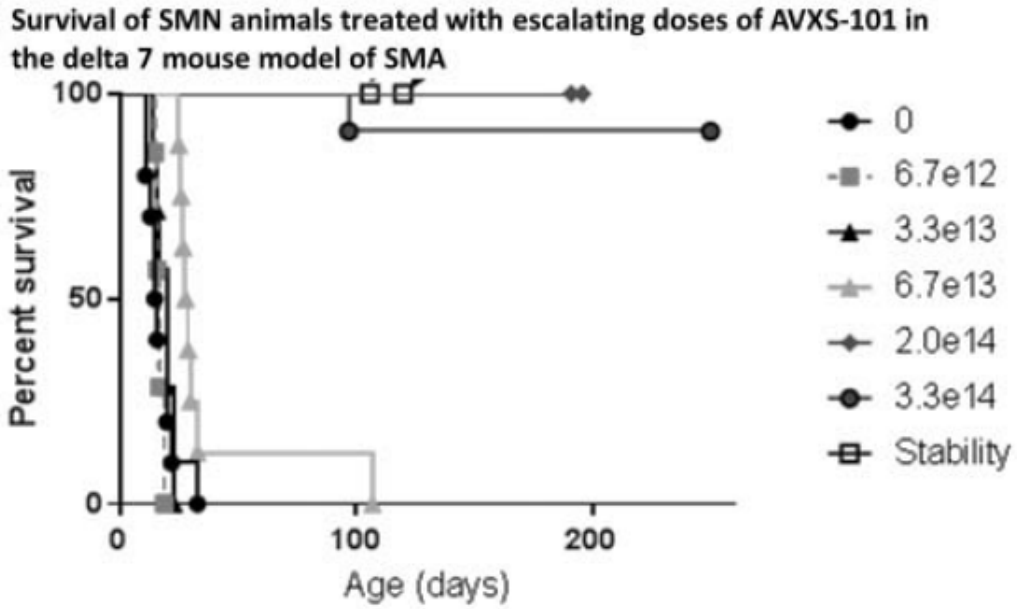


Figure 1 Survival of SMN animals treated with escalating doses of Zolgensma in the delta 7 mouse model of SMA

The above Figure 1 introduces additional efficacy data for a dose of $2.0x10^{14}$ vg/kg. The Applicant anticipated the human dose of $2.0x10^{14}$ vg/kg (when using the validated quantification assay: i.e. $1.1x10^{14}$ vg/kg) as MABEL the therapeutic effect (i.e. survival) was indistinguishable from the $3.3x10^{14}$ vg/kg dose. However, only survival was based on an analysis in only 2 mice for the dose of 2.0×10^{14} vg/kg. It seems as was this latter data obtained in an *in vivo* potency assay and added together with the data obtained in the study conducted by Foust et al., 2010. Ultimately, the therapeutic window in mice is small and there is no clear rationale or justification for clinical dose selection, since underlying data are missing.

The applicant noted that for phase 1 clinical trials, clinical dose identification was based on 2 criteria identified in animal studies: doubling Delta 7 SMA mouse life span (7.4e+13 vg/kg) and restoring full life (1.2e+14 vg/kg) in delta 7 SMA mice. The approach to establish that the Phase 3 clinical dose is equivalent to the Phase 1 clinical dose, was to first establish the stability of the Phase 1 Clinical Lot (only one Lot was used in the Phase 1 study) with the newly developed analytical methods. The same approach was used to establish the phase III clinical dose but using novel analytical methods.

The underlying data are derived from the mouse potency assay. The summary table of the results of these studies reports the median survival of ΔSMA7 mice per lot and per individual mouse. From this it could be derived that the dose which is equivalent to the proposed therapeutic dose from the Zolgensma Phase 1 Clinical Study is 1.1e+14 vg/kg, as measured by the improved PCR Assay. Safety data from pivotal toxicology studies served to establish the MTD based on mortality associated with treatment-related atrial thrombosis (The NOEL for atrial thrombosis and mortality was 1.5e+14 vg/kg, which was also the MTD) whereas doses of 3.9e+14 vg/kg showed substantial liver toxicity. The safety studies were conducted with Phase 3 Clinical Lot material produced by the proposed commercial Process B, which was appropriately qualified for Phase 3 Clinical use. Therefore, retrospectively the Applicant considered that a dose of 1.1E14 represents a sufficiently large therapeutic window for safe and effective treatment. The applicant has provided a response which is more related to the fact that there is a remarkable clinical effect and that the observed liver toxicity is also clinically manageable. The Applicant is nevertheless reminded that batch to batch consistency remains an important determinant of potency and therefore potential safety. The Applicant provided some data to support starting points for dosing in terms of

efficacy. However, the changes of production process, update of the assay to determine the actual vector dose and establishment of the MTD after start of the first clinical study make the whole clinical dose determination a difficult process to be followed. Since the current clinical dose seems effective and safety issues can be handled to a sufficient extent, this issue will not be further pursued from non-clinical perspective.

Systemic administration of Zolgensma has been conducted at PND1 and several endpoints including expression of human SMN protein, body weight, survival and motor functions were analyzed. Thereby, it could be demonstrated that a single IV injection of Zolgensma at a dose of 3.3×10^{14} vg/kg increased the SMN protein levels in spinal cord (SC), brain and skeletal muscle of SMNΔ7 mice, although similar expression levels as observed in control animals were not reached. The applicant indicated that the large inter-animal differences observed by Western blot are most likely due to normal variation within the strain. If this is indeed the case, the expression data evaluating SMN levels in SMN Δ7 mice treated with Zolgensma should be interpreted with caution as some of the observed increased levels of SMN protein in Zolgensma treated mice could be attributed to the strain variation. In addition, the applicant confirmed that the control animals are heterozygous animals expressing only 50% SMN protein as compared to wild type mice.

The relation between Zolgensma dose and efficacy upon ICV injection was investigated in the mouse model for SMA. SMNΔ7 mice receiving the highest dose of Zolgensma via the ICV route at PND1 had a median survival of 282 days. The next two lower doses (2.6×10^{13} and 1.8×10^{13} vg/kg) had median survivals of 274 and 165 days, respectively. The lowest two doses exhibited median survivals of 24 and 19 days, respectively, which did not significantly differ from untreated mice, which exhibited a median survival of 17.5 days. The lowest effective intrathecal dose appears to be 1.8×10^{13} vg/kg, whereas the clinically targeted intrathecal dose expected to provide maximal patient benefit appears 3.3×10^{13} vg/kg. Biodistribution was studied simultaneously with the AAV9.CBA.GFP construct, which appeared to have similar biodistribution as Zolgensma. With the highest dose of the AAV9.CBA.GFP vector, 46, 47 and 72% of cervical, thoracic and lumbar motor neurons were transduced respectively.

Considering the fact that treatment with Zolgensma is a one-time treatment aiming at maximal transduction of motor neurons, direct administration of Zolgensma into the CNS could yield better transduction efficiency than systemic administration at least depending on the age at treatment. The two administration routes, however, have not been compared in a systematic manner in appropriate non-clinical studies. A systematic non-clinical approach (e.g. side-by-side analysis of IV vs IT administration of Zolgensma at different ages in a relevant species) could have provide valuable information on the preferred administration route depending on the age and the applicant was asked to justify lack of such studies and to provide a justification for the chosen administration route**.**

The chosen route of administration has been justified by indicating that patients with more severe forms of SMA, including SMA type 1, are more likely to have systemic manifestations that would benefit from IV administration. Furthermore, IV administration is demonstrated to be effective in the treatment of infants with SMA in the clinic for infants treated with Zolgensma at an age of < 6 months.

Induced model of SMA in piglets, intrathecal and intracisternal dosing

Distribution of the scAAV9.CB.GFP vector and expression of its transgene was investigated in 2 groups of three 5-day old piglets that were dosed either intrathecal (at L5) or via intracisternal (at the base of the skull) injection with 5.2×10^{12} vg/kg of scAAV9.CB.GFP. All piglets were sacrificed between 21 and 24 days post injection and tissues were collected for histology and immunohistochemistry evaluation. GFP expression was observed in the dorsal root ganglia, spinal cord grey and white matter. Extensive motor neuron transduction as well as the large ventral horn neurons was observed. In the brains, highest expression levels were seen in cerebellar Purkinje cells, medulla nerve fibers as well as discrete nuclei, such as the olivary nucleus. Expression within the rest of the brain was restricted to scattered cells near

the meningeal surfaces. Absence of significant GFP expression in peripheral tissues confirms selective CNS transduction following IT and intracisternal administration.

A model for SMA in piglets was generated by transducing piglets on PND5 with an AAV9 vector carrying a short hairpin sequence to downregulate SMN1 mRNA expression (shSMN1). This resulted in a 70% reduction in SMN mRNA levels in motor neurons postnatally, which was sufficient to induce a SMA-like phenotype in the neonatal pigs that accurately mimic the characteristic electrophysiological and histological changes associated with SMA pathology in the clinic. Animals were either presymptomatically, on PND6 or symptomatically on PND33-36 transduced with Zolgensma (AAV9 vector carrying the SMN1 transgene). Immunostaining of pig spinal cord reveals a strong tropism of AAV9 for the motor neuron, with glial cells transduced only on rare occasions. Upon presymptomatic treatment on PND6 SMA disease phenotype was completely prevented (dose 8 x 10^{12} vg/kg). Upon symptomatic treatment at PND33-36, partial amelioration of the disease was observed (dose 2-3.8 x 10^{13} vg/kg. Although symptomatically treated piglets still respond and benefitted from treatment with Zolgensma, these experiments suggest that early delivery of the therapeutic vector is critical for optimal efficacy.

Although Zolgensma has been administered via the IT route only in this SMA model, potential correlation between transduction efficiencies of motor neurons and amelioration of SMA pathology in this larger SMA animal model might provide helpful additional information on the percentage of transduced motor neurons required for a potential clinical benefit.

The applicant additionally clarified that in the porcine SMA model only a single dose of Zolgensma has been tested, indicating that no dose-response relationship has been established in this model.

Cynomolgus monkey; IV and ICV dosing

Intra venous injection of scAAV9.CB.GFP in young cynomolgus monkeys led to transgene expression in skeletal muscle and organs including testes, heart, spleen and small intestine. Transgene expression in heart tissue was considerably less than in mice. In addition, injection at PND1, PND30 or PND90 led to passage of scAAV9.CB.GFP over the blood-brain barrier. If administered to NHPs at PND30 and PND90, the applicant indicated that still extensive transduction of motor neurons predicting clinical efficacy has been observed. However, no percentages of transduced motor neurons were provided. As PND30 and PND90 in NHP correspond to an age of 3 months and 1 year in human (Kim et al., 2017), these data are considered important, as treatment of paediatric SMA Type 1 patients could occur up to an age of 1.5 years depending on the body weight of the patients.

The applicant discussed the postnatal development of the human brain, thereby emphasizing that at an age of 36-months the human brain has reached approximately 80% of its volume and the number of neurons is expected to be equal to the number in adults. Thus, the applicant indicated that there may be no need for further linear vg/kg dosing at this age.

In addition, the applicant suggests that the clinical benefit is more likely related to the progressive loss of motor neurons than a change in transduction efficiencies as a result of age. While it is true that the AAV vector can only transduce neurons that are still viable, it has also been demonstrated that with increasing postnatal age, the percentage of transduced glial cells is increasing, while the number of transduced neurons is decreasing depending on the CNS structure investigated. Age may influence the transduction efficiency upon i.v. administration in a healthy animal. Data on transduction efficiency (the percentages of transduced neurons) in NHPs treated at postnatal day 30 and 90 was not present, consequently limiting the conclusions on the influence of age on transduction of neurons. However, both data in mice and cynomolgus monkeys agree with those published by two independent groups utilizing IV delivery of AAV9 to the CNS in rats and cats, provide further support for early treatment of infant patients with SMA.

Cynomolgus monkeys were dosed IT with 1 x 10^{13} vg/kg (correlating with 2 x 10^{13} vg/monkey) AAV9.CB.GFP either or not in the Trendelenburg position. Maximal transduction efficiency was achieved when animals were held in the Trendelenburg position for 10 minutes post injection. GFP expression was 55, 62 and 80% for cervical, thoracic and lumbar regions, respectively. In mice, a dose of 3.3 x 10^{13} vg/kg yielded GFP expression of 46, 47 and 72% in cervical, thoracic and lumbar regions, respectively. This suggest that with a lower dose, even higher transduction efficiency is achieved in cynomolgus monkey as compared to mice.

The pharmacology nonclinical data of Zolgensma included in the dossier have been conducted with research viral preparations. During further development of the manufacturing process of Zolgensma, manufacturing Process A has been established at the Nationwide Children's Hospital (NCH) for manufacturing of the test material used in the Phase I clinical trial. Finally manufacturing Process B has been established at AveXis, which represents the current proposed and validated commercial process. Process A material has not been tested in non-clinical studies. Process B material has been used in combined non-clinical biodistribution and toxicology studies, but not in pharmacology studies included in the dossier.
Importantly, the titration method used for determining the vector genome (vg) titer of Zolgensma has been concomitantly improved during manufacturing process development. Re-titration of previously titrated lots, resulted in varying vg titers and a retrospective re-calculation of administered Zolgensma doses. This approach raises questions on the reliability of the previously used titrations methods and the indicated administered doses in the non-clinical pharmacology studies. The applicant indicated that additional pharmacological data with Zolgensma lots representative of the planned commercial product and titrated with the ddPCR method have been performed and that a dose response for rescue of the SMNΔ7 has been established. The applicant has summarized the requested results of the in-vivo relative potency testing from 8 lots of Zolgensma representative of the planned commercial product.

Initially the dose-response in the dossier indicated that 6.7 x 10^{13} vg/kg of Zolgensma (research preparations) increased the survival time of SMA mice from 15 to 35 days, while 3.3 x 10^{14} vg/kg increased the survival to > 250 days. While it is not clear how the research preparations of Zolgensma were titrated, the Phase 1 material manufactured at the NCH has been titrated by two different methods The re-titration of this Phase I material revealed a titer of 1.1 x 10^{14} vg/kg instead of the 2.0 x 10^{14} vg/kg titer previously measured by qPCR. Subsequently, the dose needed for doubling the survival time of SMA mice has been determined as 7.4 x 10^{13} vg/kg. Finally, Zolgensma potency was measured relative to the Phase I material (used as reference material) and both the Phase I reference material and several different Phase III batches (representative of the planned commercial product) revealed comparable survival times depending on the dose administered: Thereby, a dose of 1.2 x 10^{13} vg/kg Zolgensma resulted in an increase of the survival time of the SMA mice from ~ 2 week (14-17 days) to ~ 3 weeks (20-25 days), while 7.4 x 10^{13} vg/kg Zolgensma increased the survival time to ~ 4 weeks (28-34 days). Based on these data it can be concluded that the potency of the Phase III material has been demonstrated and that the potency of this material is comparable to the Phase I material.

Secondary pharmacology

Efficacy of the product to reverse disease-related cardiac deficits was studied in neonatal SMNΔ7 mice. In the SMA mice model (SMNΔ7 mice) a decreased mass of the left ventricle and decreased wall thickness putatively due to eccentric hypertrophy is observed. Resultants are decreased heart rate and decreased heart function compared to wild-type. Treatment of these mice with Zolgensma at PND1 partially improved these symptoms as IV treatment with 5 x 10^{11} vector particles (3.3 x 10^{14} vp/kg) of Zolgensma on PND1 improved left ventricle (LV) remodelling and fully corrected heart rate. The applicant notes that both extent of dilation as well as wall thinning were attenuated and returned toward WT values in treated SMNΔ7 mice. It is not clear whether the treatment prevents occurrence of the heart related effects, which seems more plausible, or whether the effects are restored.

SMN is as a molecular scaffold onto which U small nuclear ribonucleoproteins (U snRNPs) are assembled in the cytoplasm then shuttled into the nucleus. UsnRNPs are crucial for the proper splicing of pre-mRNA to mRNA. Reduction in SMN leads to a deficiency in UsnRNP assembly and complete SMN loss is embryonic lethal in mice exemplifying the crucial role of SMN and mRNA splicing across tissues. Reduction of SMN leads to the specific degeneration of spinal motor neurons, which could potentially be explained by SMN's RNA binding properties as a potential vehicle to transport mRNA down the length of axons for distal translation, a role also suggested from research in *In vitro* models with SMN deficient neurons, indicating compartment specific reductions in mRNA translation.

Instances of naturally occurring SMN overexpression were recently reported as human cell lines derived from non-SMA individuals were shown with 3 copies of *SMN1*, which was not associated with defects. However, due to the treatment with Zolgensma, the number of copies of SMN1 may reach on average 100 – 200 fold the normal levels thereby not excluding much higher transgene levels in single cells. Risks associated with these expression levels are not discussed by the applicant. In the absence to life threatening toxicity clearly related to or associated with SMN1 expression levels, the issue of safety issues related to high transgene expression levels will not be further pursued. The applicant should keep bearing in mind that high transgene expression levels or increase in transcription and translational capacity might be responsible for liver toxicity observed in the clinic as in this organ most SMN1 copies per diploid genome were found.

Thus, SMN protein deficiency leads to early and persistent cardiac dysfunction in mice, which can be partially to fully corrected by systemic AAV9 SMN delivery. It is not understood whether this is due to the transduction of the heart muscle cells directly or whether it is a resultant of the transduction of neurons that stimulate heart contraction. Whether it is a secondary or a primary pharmacodynamic effect could be a point of discussion.

It can be agreed that no separate safety pharmacology studies or studies addressing pharmacodynamic drug interaction studies with Zolgensma need to be conducted.

2.3.2. Pharmacokinetics

Zolgensma is an engineered viral vector, containing the sequence for the therapeutic protein that has to be delivered to target cells via an Adeno Associated Virus Serotype 9. This viral capsid is a natural occurring viral capsid. This introduced therapeutic vector will theoretically not integrate into the host DNA, but stay episomal in the cell. In addition to this therapeutic transgene, the vector also contains other sequences to support sufficient transcription. Sequences to enable recombination of the vector with a wild-type AAV9, to support the development of a recombination competent virus from the therapeutic vector, are excluded from the vector or limited as much as possible. No dedicated absorption studies are conducted, which can be accepted as the product is intravenously injected and 100% bioavailability could be expected. Upon intravenous administration the virus can be readily distributed systemically. The transduction efficacy of the various type of tissues is dependent on the tropism of the type of viral particle.

(Bio)Distribution

Biodistribution and persistence of Zolgensma vector DNA as well as RNA expression of the *hSMN* transgene have been assessed in the context of a preliminary 6-months IV safety and biodistribution study in neonatal FVB mice (Study # AD49TV.7A32.BTL) using a research viral preparation characterized with non-validated analytical methods. Doses has been indicated as 6.7×10^{13} and 3.3×10^{14} vg/kg Zolgensma and tissues were collected for qPCR distribution and RNA expression analyses at 3, 6, 12, and 24 weeks post-dose.

This preliminary biodistribution study was subsequently followed by two 3-months IV safety and biodistribution studies in neonatal FVB mice using Zolgensma manufactured by Process B and characterized by validated analytical methods. Zolgensma has been administered at a dose of 2.37 x 10^{14} vg/kg (Study # 20122446), or at doses of 1.5 x 10^{14} vg/kg, 2.4 x 10^{14} vg/kg, and 3.0 x 10^{14} vg/kg (Study 8384031) and tissues were collected for ddPCR and RNA expression analyses at 3, 6/7, and 12 weeks post-dose.

Biodistribution of Zolgensma, as analysed in both the 12 week and the 24 week murine GLP studies, showed a persistent and widespread and high tissue transduction upon IV administration. Also, a tissue-wide and high transgene expression (including the heart, liver and CNS) was observed, with vector distribution to transgene expression ratio in peripheral tissues ranging from approximately 1:1 to 1:10. This was also expected because of the use of a strong ubiquitous promoter used for driving transgene expression. Persistent vector and transgene levels in brains and spinal cord indicate successful crossing of the blood-brain barrier (BBB) by the vector after systemic administered to neonatal mice. Lower tissue vector distribution and transgene expression was found in a.o. spleen and gonads. This biodistribution pattern was comparable between the different doses tested. However, there was potentially lower transgene expression at week 12 for the lowest dose tested (1.5x10^{14} vg/kg), indicating that -despite persistent vector transduction- expression of the transgene may wane over time. Thus, highest levels of vector DNA and hSMN RNA expression has consistently been observed in the heart followed by skeletal muscle, SC, brain, liver and lung, whereas relatively low levels of vector DNA and hSMN RNA expression have been observed in spleen, testes and ovary.

Generally, it would be expected that a complete list of organs and tissues is analysed for the biodistribution of the viral vector. The provided justification of the applicant indicates that the analyses of the organs and tissues has been restricted to the organs either involved in the pathology of the disease or expected to be highly transduced based on the vector tropism. As the tropism of the viral vector is not exclusive for the indicated organs, a less restricted biodistribution analysis would be preferred. Since a complete list of organs/tissues has not been investigated, it has to be assumed that the viral vector is also reaching other organs and tissues that have not been included in the analysis, and that the transgene may also be expressed in these organs/tissues. This assumption is supported by the two clinical autopsy reports from 2 deaths (occurring 52 days and 5 months after vector administration, respectively). The autopsy reports also listed the distribution of the viral vector and the transgene expression in the following organs/tissues: brain (different regions in one of the two deaths), spinal cord (different levels), dorsal and ventral roots, DRG, diaphragm, skeletal muscles, thymus, small and large intestine, stomach, lung, pancreas, kidney, heart, spleen, liver, and lymph node. In almost all of the investigated organs and tissues including kidney the vector DNA could be detected and expression of the transgene was detectable (mRNA or protein).

Based on the available biodistribution data from two treated infants that died during the phase III studies the kidney seemingly is an target organ for the viral vector. Therefore, biodistribution data of viral vector to the kidney in the mouse would have been interesting, but no new animal studies are requested.

The persistent presence at week 12 of high vector DNA and transgene mRNA levels in various tissues (primarily heart and liver) in mice may indicate that the adverse events found in the 12 week GLP studies are probably related to the presence of (lots of) vector DNA and/or ubiquitous (over)expression of the transgene, which is discussed in the toxicology section. In NHP, a similar wide-spread vector distribution and transgene expression was observed, both after IV or IT administration, although the level differed for some organs compared to mice (e.g. NHP = lower heart transduction). Therefore, the toxic effects observed in mice with Zolgensma ca could potentially be clinically relevant.

The Applicant has expressed their biodistribution study results as vector copies per µg gDNA, which provides an indication of the amount of transduction of the whole tissue. Additionally, vector transduction

could be expressed as copies per diploid genome. This would provide more insight in the transducability of tissues at a single cell level (enabling comparison between different studies) and would allow for estimation of the risk on cellular stress response after transduction with large numbers of vector genomes and/or transgene overexpression. These data could also have provided useful information to explain (part of the) toxicity, e.g. in the heart. Upon request the data were not provided, but instead human Zolgensma distribution data expressed as vector copies per diploid genome from one patient who died (study CL-303) were provided. Also, reference was made to distribution data from another patient (study CL-302) who died, was presented in the clinical part of the dossier, which was more limited with regard to number of tissues analysed. Whether these profiles are a general reflection of all patients is unknown. In addition, biodistribution at single cell level could be different in mice compared to humans. For example because:

- fatal atrial thrombosis occurred in mice, but -according to the Applicant- no cardiovascular safety issues were present in human patients which may indicate a difference in transducability at individual cardiomyocyte level between mice and human;
- transduction of murine spleen was consistently lower in the different toxicity studies compared to the other tissues analysed, while the vector copies number in humans is considerably high (average = 10 copies per cell); this could also have resulted in a higher vector copy in the whole tissue (here: spleen) compared to mice.

The Applicant provided an overview of all available human whole tissue and single cell biodistribution data in comparison to whole tissue murine biodistribution data (from the GLP 12-week studies) to determine similarities and differences in transducability per tissue between mouse and human.

The Applicant has not determined shedding of the vector, for example in serum, saliva, tears and sweat, while this is regarded integral part of biodistribution studies. Nevertheless, in the clinical part of the dossier, human shedding data of AAV9 (based on literature studies) is provided, which is considered sufficient.

In addition, testes in NHP were positive for vector DNA and transgene RNA, although the cell type transduced (and thus potential risk of germ line transmission) was not determined. No data regarding female ovaries were available. In mice, an unexplained higher SMN expression was found at the highest dose ($3x10^{14}$ vg/kg) in female ovaries at week 12 compared to week 3 and 6 (study no. 8384031).

Four different methods used for determination of vector DNA into the tissues and determination potential formation of antibodies are described. Three of them are also used in the clinic and one is only used for animal studies. The methods seems fit for purpose.

It can be anticipated that the AAV9 capsid after entrance of the cell will be broken down via normal cellular routes. The building blocks for DNA and the therapeutic protein could also be regarded as natural to human. Thus, absence of metabolism or excretion studies or studies addressing pharmacokinetic drug interaction studies can be agreed because of the biological nature of the product.

The applicant indicated that no tissues were to be judged as negative. Indeed, Biodistribution data included in the report of Study AD49TV.7A32.BTL revealed mostly positive results for vector DNA for the investigated organs and time points of animals administered Zolgensma. The only exceptions were the gonad samples from Week 24, which turned out to be either negative or containing vector DNA levels ranging between 49 and 128 copies/µg gDNA. These data reveal the following: 1) negative signals were only detected at the latest time point investigated, and 2) only samples revealing ≤ 50 vector copies/µg gDNA were declared as negative.

2.3.3. Toxicology

The Applicant has provided a pilot study and three GLP studies in which the safety and biodistribution of IV-administered Zolgensma was tested in neonatal wild-type mice. Furthermore, a pilot IV study in NHP which were dosed at PND90, was provided. All studies were performed with pre-process A batch material, except for the two 12-week GLP studies (process B). Additionally, two studies in mice and one study in NHP were provided, which determined safety and biodistribution of Zolgensma after ICV/IT injection. However, these were all non-GLP studies conducted with a research grade batch (pre-process A) for which vector quality and titre were analysed with non-validated methods.

In all murine toxicology studies, the Applicant has used wild-type FVB/n mice. These albino mice appear to become blind at weaning age, but also seem to have decreased Integrin α2 expression leading to decreased platelet adhesion to collagen, which in turn results in decreased thrombus formation. This strain is not often used for toxicology studies. It is thus not known whether there is sufficient historical control data, which hinders distinguishing the product-related findings from the strain background-related findings. It is noted that this strain was also used as background strain for the SMA mouse model (SMNΔ7 mouse).

The Applicant has chosen to use the FVB/n strain in the toxicology studies to be able to directly compare safety data from the SMNΔ7 studies. However, these studies were performed with research batches (pre-process A), while the GLP studies were conducted with process B batches. Because manufacturing process B is most likely considerably different from pre-process A and because different doses (i.e. at least determined with a different assay) have been used in the pharmacology and toxicology studies, a direct comparison between data from both study types would not have been perfect either. As the FVB/n background was needed to create the SMNΔ7 model and no historical background data would be available for this strain when injected with Zolgensma at PND0/1 in the temporal vein, the rationale for choosing the same strain for toxicology studies is considered sufficient. Although the targets of toxicity are clear, the level of toxicity (leading to calculation of the NOAEL/MTD) might have been underestimated using this specific strain. As the Applicant considers monitoring liver, heart and coagulation important in the clinical trials, these potential underestimated safety issues might be detected early enough to enable intervention, upon occurrence.

The homology between NHP and human is higher than between mice and human. Nevertheless, the NHP raised an antibody titre against both the vector and the transgene, while the mice raised an antibody titre against the vector alone. The Applicant considers the age of the animals species at treatment most important to explain this difference.

Mice IV administered with Zolgensma (Pivotal studies)

The Applicant conducted two 12 weeks and one 24 weeks GLP compliant studies with a single IV Zolgensma administration in neonatal FVB wild type mice as indicated in the table below.

Table 2 Mice studies administered with Zolgensma IV

Study ID	Species/ Sex/Number/ Group	Dose/Route	Observed max non-lethal dose/MTD	Major findings
AD49TV.7A32. BTL	Neonatal WT mouse M and F 22-23/sex/group (week 3) 6-9/sex/group (week 6, 12, 24)	0 6.7×10^{13} vg/kg 3.3×10^{14} vg/kg*	Observed max non-lethal dose: 3.3×101^{4} vg/kg*	None
20122446	Neonatal WT mouse M and F 5/sex/group	0 7.9×10^{13} vg/kg 2.37×10^{14} vg/kg 3.91×10^{14} vg/kg	Observed max non-lethal dose: 2.37×10^{14} vg/kg	Cardiovascular toxicity (atrial thrombosis)
8384031	Neonatal WT mouse M and F 5/sex/group	0 1.5×10^{14} vg/kg 2.4×10^{14} vg/kg 3.0×10^{14} vg/kg	MTD: 1.5×10^{14} vg/kg	Cardiovascular toxicity (atrial thrombosis)

* Research viral preparation used as test material that has been manufactured with a different manufacturing process and not characterized by the current validated titration method (ddPCR); the actual administered vector dose might differ from the indicated dose.

As indicated in the table above, Study AD49TV.7A32.BTL was the only GLP safety study that did not reveal major toxicity findings at doses up to 3.3×10^{14} vg/kg Zolgensma. However, this study has been conducted with research viral preparation using a manufacturing process that preceded the establishment of Process A and appeared not to be a pivotal, GLP compliant study. For the test material used in Study AD49TV.7A32.BTL, HEK293 cells were transfected in roller bottles. After lysis, the preparation was subjected to two rounds of CsCL ultracentrifugation, with the collected band of full capsids dialyzed into formulation buffer containing poloxamer 188.

The subsequent GLP single dose toxicity studies, Study 20122446 and Study 8384031, using GMP test material from the initial Process B manufacturing process revealed dose-dependent, delayed cardiovascular toxicity that was associated with thrombus formation in the atrium of the heart resulting in unscheduled mortality in a significant number of animals.

The first 12 week study performed with a GMP-compliant batch from process B (study no. 20122446) resulted in a systemic inflammatory response at week 3 in all dose groups (i.e. 7.9×10^{13}, 2.37×10^{14} and 3.91×10^{14} vg/kg), followed by signs of blood loss or stress in the two highest dose groups at later time points. Additional clinical abnormalities (e.g. hypoactivity, breathing difficulties, abdominal distension, closed or dark eyes) and unscheduled mortality (100% before week 12) were only present in the highest dose group. The cause of death in 13 of these mice was due to atrial thrombi, but for the other 16 mice no cause of death was determined, although heart and liver lesions were found. This requires further discussion (see below). Comparable lesions were found in mice that survived until scheduled death, although lesion severity was dose-dependent and (partly) resolving over time. Degeneration/regeneration in heart was considered minimal in severity at a dose of 7.9×10^{13} vg/kg, and minimal to mild in severity at doses of 2.37×10^{14} vg/kg and 3.91×10^{14} vg/kg, respectively. Similarly, minimal signs of regeneration were observed in liver of females administered 2.37×10^{14} vg/kg AXVS-101 and in the liver of both males and females administered 3.91×10^{14} vg/kg Zolgensma. The NOAEL in this study was 2.37×10^{14} vg/kg.

The second 12-week study performed with a GMP-compliant batch from process B (study no. 8384031) showed adverse events and unscheduled mortality in all dose groups tested (i.e. 1.5×10^{14}, 2.4×10^{14} and 3.0×10^{14} vg/kg). The Applicant was asked for a clear (tabulated) summary of the mortality rates in the different groups, including their cause-of-death and/or main pathological observations (see remarks on

studies below). Part of the mortality cases (i.e. 11 out the 19) were product-related, based on clinical observations (a.o. hypoactivity, breathing difficulties, protruding/dark eyes), changes in blood parameters (indicating stress, inflammation and/or dehydration) and pathological abnormalities (dose-dependent lesions in ventricles, atria, liver, lung and spleen/thymus). Death was associated with atrial thrombosis, sometimes accompanied by atrial dilation, fibroplasia, myocardial degeneration/necrosis, and/or mononuclear inflammation. Atrial thrombosis and atrial wall changes were dose-dependent and present in mid and high dose animals (≥ 2.4 x 10^{14} vg/kg). Although the Applicant states that 8 of the 19 premature deaths were likely not related to Zolgensma, it may be related to injection at PND0 instead of PND1 used in the other studies. All dose groups showed reduced food consumption and body weight (gain). Although potential trends towards differences in blood parameters might be present, lack of data from sufficient animals prevented further assessment of these values. Histopathologically, product-related lesions in a.o. the liver (hepatocellular hypertrophy) and ventricular wall of the heart (oedema, inflammation, fibrosis) were found in all dose groups, while atrial wall changes (fibroplasia, degeneration/necrosis, dilation) and thrombi, inflammation in the lung and more severe liver lesions (e.g. necrosis and vacuolation) were only found in the intermediate and high dose groups. Based on the lack of similar cardiovascular toxicities resulting in Zolgensma-related mortality, the low dose of 1.5 x 10^{14} vg/kg was defined as the maximum tolerated dose (MTD). The no adverse effect level (NOAEL) could not be defined in this study, based on the liver and heart lesions that included minimal hepatocellular hypertrophy, minimal ventricular myocardial oedema, minimal myocardial fibrosis, and minimal mononuclear cell inflammation that were observed also in the low dose group. These effects were of low severity and not life-threatening and are probably directly associated with the administration of AAV vectors and subsequently induced inflammatory responses.

The underlying mechanisms of atrial thrombosis and mortality observed in neonatal mice after IV administration of Zolgensma are not known at the time and the applicant did not indicate whether and how these non-clinical findings will be further investigated. It is acknowledged that available clinical cardiovascular safety data have not provided evidence for severe cardiovascular safety problem in human. However, the safety of Process B material has not yet been sufficiently demonstrated.

The applicant only considers the two 12-weeks study as relevant for the toxicity observed in the heart and made a distinction between ventricle and atrium related findings. Adverse findings related to the ventricles (no NOAEL) comprised of mainly inflammation, followed by oedema and fibrosis, likely attributable to the prominent biodistribution (and related immunogenic response) of Zolgensma to the heart, but were only minimal to slight in severity and would not be considered to be life threatening. Strikingly, no thrombosis was seen upon ventricle inflammation in contrast to atrium inflammation. This is not notified by the applicant.

Findings related to the atrium were dose-related and present at doses of ≥2.4E14 vg/kg and the NOEL for atrial thrombosis was 1.5E14 vg/kg and often cause of death or reason for unscheduled sacrifice. According to the applicant the most likely pathogenesis of atrial thrombosis is considered to be inflammatory or degenerative injury to the endothelial/subendothelial surface of the atria exposing a thrombogenic surface and precipitating atrial thrombosis. However, this hypothesis is only theoretical in nature and not supported by any non-clinical data.

According to the applicant, no alteration in blood coagulability was present and could thus not contribute to atrial thrombosis. However, in 12-week study no. 8384031, a trend towards increased coagulation times (at PND 84) and decreased platelets (at PND 21-84, depending on the sex) could be observed in the highest dose groups, although not all parameters were significant due to limited animal numbers. In addition, in 12-week study no. 20122446, the Applicant stated that a decrease in platelets was observed in pre-terminal animals and that this decrease "may have been related to Zolgensma and associated with thrombus formation in the heart" (page 898 of the report). An AAV-related systemic inflammatory reaction could have resulted in platelet activation with subsequent intravascular coagulation at

thrombogenic surfaces and thrombocytopenia (e.g. Hinderer et al. 2018). Alternatively, AAV-induced complement activation and subsequent opsonisation of platelets could have resulted in altered coagulability.

The applicant did not regard the expression level of SMN in the mouse upon Zolgensma treatment to be causative for the atrial thrombosis. The applicant did not consider the overstretching of cellular functioning (transcription and translation machinery) due to the high viral transduction as a potential cause of thrombosis.

The applicant notes that translatability of this finding to humans is not known, but that upon identification of the heart as toxicity target organ, adequate cardiovascular safety monitoring measures were put in place in the clinical studies. This approach is supported. In relation to the translatability of the findings, the applicant presented data from a fatal case in clinical study 302, showing widespread viral transduction to CNS as well as to liver and heart, without inflammation or other notable findings in the heart or liver. However, the applicant did not fully exclude translatability of the finding as the applicant notes that if translatability would occur, it would likely be transient, consistent with the transient nature of liver and myocardial enzyme elevations seen in clinical studies.

The relationship of the occurrence of cardiovascular toxicity with the mouse strain used and the age of the animals at start of dosing are not clear. Insight in mechanisms underlying atrial thrombosis are limited. The occurrence of cardiovascular toxicity in the clinic should be addressed in the clinic as extrapolation from non-clinical data is not possible.

In addition to both 12-week studies, the 24-week study (study no. AD49TV.7A32.BTL) did -according to the Applicant- not result in product-related adverse events, including unscheduled mortality. However, statistically significant (dose-related) reduced body weight, changes in erythrocyte-related parameters (decreased erythrocyte count, Hb and HCT, increased MCV and MCH), changes in different leukocyte counts (decreased lymphocytes, eosinophils and basophils, increased monocytes and neutrophils) and decreased serum proteins were observed for both sexes in the vector-treated groups compared to the control group. Although these effects were temporary, they all occurred primarily in the first weeks post-injection and a relation with vector treatment cannot be excluded.

It was noted that this study was performed with a research batch (pre-process A), while the 12-week studies were performed with a clinical grade batch (process B). In addition, for dose quantification in the 24 week study no validated PCR method was used. Therefore, no conclusions can be drawn based on this study and thus no long-term study data (> 12 weeks) are available for assessment.

The difference in toxicity outcome for the three GLP studies is significant, varying from no adverse events (according to the Applicant, 24 week study no. AD49TV.7A32.BTL) to adverse events at the lowest dose used (12 week study no. 8384031) or 100% premature mortality before final sacrifice in the highest dose group (12 week study no. 20122446). Although three different batches have been used (and potentially also different doses), their purity and sterility does not seem to differ and both 12-week studies have been performed with GMP-compliant batches (using process B).

Apparently, results from the 24-week study can not be compared with the 12-week murine studies, because the exact dose could not be established, and the study was conducted with a pre-process A batch. Actual toxicity data that could be relevant for the clinic should therefore come from the GLP 12-week studies. Indeed, main toxicity targets are liver and heart. But the severity of the clinical signs and toxicity-related mortality is different between both 12-week studies.

The Applicant has provided information regarding the (different) pathology terminology used in the pivotal 12-week studies. However, since a pathologist is expected to be able to differentiate between degeneration and subsequent regenerative signs (present in study 20122446, but not in study 8384031), the difference in study outcome with respect to lesion reversibility is not considered a matter of

terminology. In addition, mortality is not regarded related to terminology. For example, a considerable difference in the outcome of the studies with comparable dosing is that all animals in the highest dose group of study 20122446 (i.e. $3.91x10^{14}$ vg/kg) did not survive until week 12, while most of the animals in the highest dose group of study 8384031 (i.e. $3.0x10^{14}$ vg/kg) did survive until week 12. In contrast, no obvious clinical signs or mortality were present in the mid-dosed group of study 20122446 (i.e. $2.37x10^{14}$ vg/kg), while adverse clinical events (e.g. reduced body weight) and mortality in study 8384031 already occurred in the low-dosed group (i.e. $1.5x10^{14}$ vg/kg) and thrombus-related mortality was present in the mid-dosed group (i.e. $2.4x10^{14}$ vg/kg). Taken together, distinct outcomes are present in both studies. The Applicant discussed that the unscheduled deaths that did not have significant atrial lesions and/or thrombi (e.g. 16 mice in the highest dose group of study no. 20122446) that were not atrial thrombus-related. The other heart and liver lesions were not considered different between unscheduled and scheduled deaths and would therefore not be related to the cause of death. The Applicant states that the FVB/n strain may be very susceptible to mortality, although this was not further substantiated with other (publicly available) data. This could be an explanation for the considerable numbers of unscheduled deaths, in combination with the very young age of the animals at treatment.

Differences in frequency and severity of adverse events (i.e. in study no. 8384031) between the two sexes could be caused by increased susceptibility of males to Zolgensma-related toxicity. Although not mentioned by the Applicant, stress due to housing with non-littermates (aggression males > females) may also play a role, but this is not treatment-related. It was noted that animals in study 8384031 were weaned at day 21, while the animals in study 20122446 were weaned 1 week later. In addition, animals were housed individually (study 8384031) or with maximum 3 animals of the same dose group (study 20122446). This difference in weaning and housing strategy may also have influences the stress level (and thus susceptibility to adverse events) of the animals in both studies.

Haematological and clinical chemistry analyses were started only 3 weeks after the first dose, while adverse events in humans, e.g. thrombocytopenia and increased transaminases, occur in the first weeks post-injection. Apparently, an earlier time point for drawing blood from the mice was not technically feasible. Early clinical findings could therefore not be related to findings in the NC toxicology studies. The applicant states that the pathogenesis of the toxic events could be related to the vector and/or the transgene. However, neonatal administration of Zolgensma most likely induced immune tolerance for the transgene (NB: absence of antibody responses to the transgene were only evaluated with pre-process A batches). Moreover, the Applicant concludes that transgene overexpression should not result in adverse events, because it is a physiological status in some people to have several copies of SMN1 (although Zolgensma may result in many more vector copies and thus also transgene copies in specific tissues). It is therefore still unclear whether the adverse events are the consequence of vector-related, transgene-related and/or species-specific effects. Because there are also effects on the liver and heart in human patients treated with Zolgensma, at least the (immune response against the) vector, high transgene copies (i.e. cell stress-related cell death) or foreign transgene copies in specific tissues are expected to play a mechanistic role in the adverse events. Difference in severity of the toxic events between mice and humans may be related to differences in transducability of certain tissues and/or the use of corticosteroids.

The clinical relevance of the observed Zolgensma related effects in animals, with special attention to occurrence of tissue lesions (primarily liver and ventricle) already found in doses 0.7- to 1.4-fold the clinical dose and the striking mortality in mice at doses 2-3 the clinical dose required discussion. Apparently, most important Zolgensma related toxic events in mice occurred in the liver and heart (both ventricle and atrium). The Applicant has mentioned that liver tissue is highly transduced in both mice and human and that liver lesions also occur in the clinic (i.e. increase in transaminases), although transiently (most likely because of the use of corticosteroids). The Applicant considers the toxic events in the mice as being vector-related. Because this is a platform effect and the heart is also significantly

transduced in humans (at least at single cell level, see Question 162), the change on toxic heart issues in the clinic should not be underestimated. An appropriate monitoring system for the functionality of vital organs and a functional intervention protocol when (life-threatening) toxic events would occur should be in place. This is further assessed in the clinical sections.

Upon request, the applicant provided a discussion on whether the very young age of the treated mice could be associated with the induction of the observed atrial thrombosis. Although it cannot be ruled out that it could have an influence, the applicant emphasized that atrial thrombosis peaked between 3 and 7 weeks post-dose, a time-point when coagulation and haemostasis is functionally matured and the mice reached an age equivalent to a child or adolescent in human. Thus, in the applicant's view the time course of the observed atrial thrombosis rather suggests that the immune response to foreign AAV capsid and/or expressed transgene product antigens and the lymphocytic myocardial infiltrates result in inflammatory or degenerative injury to the endothelial/subendothelial surface of the atria and subsequently to atrial thrombosis. Such a scenario would not be age-dependent. The applicant further sufficiently justifies the very young age of the mice chosen for Zolgensma administration, which is a.o. due to vector distribution and BBB development. However, for investigating whether there is a correlation between the very young age of the mice at treatment and the occurrence of atrial thrombosis, the vector administered IV may be inducing the observed cardiovascular toxicities. Thus, such studies could have been conducted in mice. Thus, the provided justification for not investigating this aspect non-clinically falls short. However, as the answer of the applicant reveals that there are at least 6 SMA patients that were treated at an age of 8 – 14 days and since the available clinical safety data as of 22 March 2019 show no cardiovascular adverse events that have been judged to be related to Zolgensma, this issue is no longer pursued.

Part of clinical chemistry data (or measurement in only one or two animals), coagulation assessment in the intermediate (females) and high (males) dose groups in study no. 8384031 was lacking. The provided justifications are considered acceptable. The applicant noted that clot formation in the blood samples was related to atrial thrombi. As the clot formation took place during/directly after blood collection, this conclusion indicates an increased state of blood coagulability. However, the applicant states that atrial thrombus formation is not very likely caused by alterations in blood coagulation capacity. The Applicant should note that an increased state of blood coagulation capacity may still be (part of) the cause of atrial thrombus formation. In addition, clot formation may be caused by an inappropriate ratio between blood volume and anti-coagulant in the collection tube.

Taken together the design of the pivotal studies with dosing at very young age and the infeasibility to evaluate parameters early after dosing, it appears that the relevance of these murine studies for the clinic, regardless the study outcomes, is only marginal.

NHP IV administered with Zolgensma (non-GLP)

In a non-GLP study, male cynomolgus Monkeys were IV administered with a relatively low dose of $6.7x10^{13}$ vg/kg of an unknown batch (with vector titre determined via non-validated method). No product-related toxicity was observed, but part of the data (e.g. necropsy) was missing, hampering proper assessment of the results.

Nevertheless, in a recently published study with a single IV injection of $2.0x10^{14}$ vg/kg AAV9-like vector carrying the SMN transgene, significant systemic inflammation, liver toxicity and neuronal degeneration were observed in juvenile rhesus macaques (with premature sacrifice of one animal) and neurological degeneration was found in piglets (all prematurely sacrificed; Hinderer *et al*., Human Gene Therapy, 2018) and the authors conclude that this is a platform-related effect. These effects may have been missed in the NHP study with Zolgensma due to the lower vector dose and/or batch type.

Due to the limitations of this study, e.g. the relatively low dose (0.6-fold the proposed clinical dose and >3-fold below the dose used in the murine GLP studies where overt toxicity was noted), the lack of

ascending dose design, the quality of the used batch (research grade), and the shortcomings in the (reported) data, this study is not regarded of additional value for the estimation of the clinical risks for Zolgensma. As in mice a dose of ~≥ 2.2 fold the clinical dose results in mortality, a safety study in an animal species more close to human would have been helpful to determine the safety profile of the product in relation to the dose. As currently human data from clinical studies are available, these data are preferred for determining the safety and efficacy profile of the product.

Mice and NHP ICV/IT administered with Zolgensma (non-GLP)

The Applicant conducted a 12 week and a 24 week non-GLP study, in which mice were administered a single ICV dose of $3.3x10^{13}$ vg/kg AXVS-101 aiming at more effective distribution to spinal cord and brain. No product-related adverse events occurred in these studies. This local treatment suggests a reduced systemic exposure as it results in a lower antibody titre compared to the 10-fold higher IV dose. However, (systemic) distribution data in mice upon ICV administration is missing.

A limited endpoint non-GLP single dose toxicity study was conducted in male cynomolgus monkeys aged 4-6 months. Animals received an IT administration of $2x10^{13}$ vg/kg Zolgensma and on the limited observations made, there were no apparent effects on body weight or haematology. One notable serum chemistry finding was in one animal receiving test article, in which ALT levels were markedly increased at 16 months post injection without histopathological correlates. This finding resolved at 18 months. In 2 test animals, platelets were markedly increased. This finding was transient and resolved after 6 months. There were no remarkable histological changes that could be attributed to administration of Zolgensma. However, systemic transgene expression (a.o. in heart, liver, lung and skeletal muscles) and substantial antibody titres (approximately 1:1600) both against the vector and the transgene were measured. Also, in the published pharmacodynamic data, systemic exposure to Zolgensma upon IT administration of $1x10^{13}$ vg/kg Zolgensma to 1 year old NHP was observed (Meyer et al., 2014, see section 2.1.7). These observations suggest systemic distribution of Zolgensma upon IT administration. No vector- or transgene-specific T cell responses were found in the IT NHP safety study.

These explorative studies may show a potential for treatment of (older) patients with Zolgensma via ICV or IT administration. Nevertheless, several limitations to these studies preclude translation to the clinic or bridging IV to ICV/IT for potential future applications: 1) these studies were performed with a research batch (no. batch SMN-131002, pre-process A); 2) vector dose was determined with a non-validated PCR assay; 3) only a single vector dose instead of dose range was analysed (thus, no dose-response could be established); and 4) in the NHP study, only male animals were used. However, this also means that the results, suggesting absence of toxicity, cannot be extrapolated to the clinic with any certainty.

Mutagenic and/or tumorigenic risk

Neither studies on vector integration nor a justification for omission of such studies has been provided in the dossier. As Zolgensma is a non-integrative vector, it can theoretically integrate into DNA to a very low extent. Consequently, the risk for mutagenic and the tumorigenic potential is regarded to be very limited. The justification for not addressing vector integration in non-clinical studies is based on the fact that only a small portion of AAV vectors integrates into the genome, while most of the DNA remains episomally. If the vector integrates, it occurs random into regions of chromatin/DNA accessibility, without a preferential integration into critical sites. So far, no cases of insertional oncogenesis have been reported for AAV vectors. Although most of the clinical experience has been obtained upon treatment of adults, there are currently no hints suggesting that infants might be more susceptible to AAV-induced insertional oncogenesis.

Transfer of the vector to offspring

In NHP testis was positive for vector DNA and transgene RNA, although the cell type transduced (and thus potential risk of germ line transmission) was not determined. Moreover, no data regarding female

ovaries were available. In mice, an unexplained higher SMN expression was found at the highest dose ($3x10^{14}$ vg/kg) in female ovaries at week 12 compared to week 3 and 6 (study no. 8384031). Vector could theoretically distribute to offspring when it is present in the gametes. The low but persistent levels of vector DNA observed in gonads should have triggered further non-clinical analyses in accordance with the Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005).

To justify the lack of germline transmission studies, the applicant discussed in detail existing literature data on inadvertent germline transmission of AAV vectors. Most of this data was generated with the AAV2 serotype and indicate that the AAV vector DNA, although distributed to gonadal tissue and shed into the semen, does not transduce sperm cells and does not result in germline transmission in animal studies. However, other serotypes than AAV2 might have a different tropism for sperm cells and oocytes. On the other hand, AAV serotypes with high tropism for gonads have not been described so far, and the referenced literature addressing different serotypes demonstrated that the kinetics of vector clearance of AAV2 and AAV8 vectors from semen was dose- and time-dependent, but serotype-independent. This suggests that data generated with other serotypes could be transferred to other serotypes at least to some extent.

As the biodistribution data of Zolgensma revealed relatively low levels of vector DNA present in the gonads, there are no hints indicating that the biodistribution of the AAV9 serotypes would largely differ from other serotypes such as AAV2 or AAV8. Based on these considerations and the fact that SMA1 patients are treated at a very young age, it may be acceptable that no further non-clinical studies were conducted on inadvertent germline transmission.

The risk for transfer of a non-integrative, non replicative vector to offspring via spermatocytes is low. As AVXS101 is modified AAV vector that can in theory not multiply itself, it is anticipated that in case spermatocytes or its progenitors are transduced with the product, that the vector will be diluted out. Seen the extended time period between treatment (preferably before the age of 6 months) and the reproductive age, the risk for transfer via male patients is regarded negligible. As oocytes are all present before birth and will start to mature in females during the period that they have reproductive potential, transduction of oocytes could potentially lead to transfer of the vector to offspring. It can be anticipated that the vector will readily dilute out in the developing embryo and may be transferred only to a limited number of cells in the final foetus. However, the potential risk for the offspring may be rather related to potential supraphysiological transgene expression levels that could putatively (dependent on the role of the transgene) interfere with early embryonic/foetal development. To that end, the applicant discussed potential interference of SMN overexpression with early embryonic development. Because SMN levels appear to be higher in embryos compared to adults (although the levels compared to neonates have not been mentioned), presence of Zolgensma related SMN copies are not expected to impact embryonic development. As *smn1* expression is low in the ovaries following Zolgensma expression, levels in a fertilized oocyte or very early developing embryo may not largely increase the level of *smn1* already present in this very early stage developing embryo. Due to the continuous cell divisions, it can be expected that the vector will readily dilute. It can thus be agreed with the Applicant that increased *smn1* expression will not pose a marked risk during very early stages of embryonic development.

Of note; the Applicant mentions that SMN protein levels in the ovaries are transient. Furthermore, the Applicant mentions that patients surviving until reproductive age will likely not have transgenes in their oocytes. As these cells are resting until puberty, it is unclear why transgene would not persist in these cells.

2.3.4. Ecotoxicity/environmental risk assessment

2.3.4.1. Hazard identification

Hazard identification identifies relevant characteristics of the medicinal product that may cause a harmful effect on human health or the environment. Furthermore, the hazard identification evaluates the potential consequences of the identified possible harmful effects, in case they occur.

The AAV vector Zolgensma

The genetically modified vector Zolgensma is derived from AAV. The viral vector is replication deficient due to the absence of the *rep* and *cap* genes. These deletions do not lead to a harmful effect such as increased persistence or invasiveness. On the contrary, they lead to reduced persistence and invasiveness since the viral vector is replication deficient because of these deletions. In view of the normal function of the SMN protein in humans, there are no reasons to assume that the SMN protein has effects on the virus biology of AAV or that the protein can complement the functions of the *rep* and *cap* genes. Expression of the SMN protein by the viral vector therefore cannot lead to the formation of new virus particles. Because the capsid of the virus particle consists of AAV9 proteins, the host range and tissue tropism will be identical to AAV9. In neonate mice, administration of 1.5×10^{14}, 2.4×10^{14} or 3.0×10^{14} vg/kg Zolgensma via intravenous injection showed widespread biodistribution of the genome and expression of the construct at 3, 6 and 12 weeks post-dosing, including in the brain and spinal cord, which indicated the vector was able to cross the blood-brain barrier when administered systemically. The highest levels of vector genome were detected in the heart, liver, lung and skeletal muscle. Similar to previous biodistribution studies with Zolgensma, low levels of vector genome were observed in the spleen relative to other tissues. A low level of vector genome and expression was also noted in the testis and ovary.

In a second study, neonatal mice were injected intravenously with 6.7×10^{13} vg/kg or 3.3×10^{14} vg/kg. The presence and persistence of viral DNA at high copy number was demonstrated in the brain and spinal cord at all post injection intervals (3, 6, 12 and 24 weeks post injection) though the highest copy number was present in heart. High copy numbers were also noted in liver, lung, lymph node and muscle. The lowest levels were detected in gonad samples and intermediate levels were measured in the spleen, pancreas, kidney and jejunum. RNA expression of the SMN transgene was demonstrated in brain and spinal cord, the targeted therapeutic tissues. In addition, human SMN was consistently expressed in the kidney, lung, heart, liver and muscle. The gonad was the only tissue in which no expression was detected in either males or females across all time points, which indicates the integration of the transgene in germline cells is highly unlikely. Low levels of circulating antibodies to the AAV9 capsid were found after 12 and 24 weeks in mice given 3.3×10^{14} vg/kg. However, no circulating antibodies to the SMN transgene were detected in any of the mice in the study, regardless of treatment group or time point.

Potential hazards to human health

Potential hazards to human health are related to (a) shedding of AAV vector particles, (b) the formation of rcAAV or recombinant viruses during manufacturing or after infusion and (c) insertional mutagenesis.

Hazards related to shedding of vector particles

Unintentional exposure of non-target individuals to the GMO may result in expression of the normal human SMN protein and induction of an immune response against the Zolgensma capsid proteins.

Hazards related to complementation, recombination and rcAAV formation

Vector mobilisation may occur in a helper virus infected patient because of the generation of rcAAV during manufacture or because of complementation and recombination between the viral vector, wildtype

AAV and a helper virus. Dispersal (dissemination) of AAV is not documented definitively, but is likely through inhalation (aerosolized droplets), contact with mucous membranes (eyes, nose and mouth) and fecal-oral transmission.

Hazards associated with recombination of the CMV and SV40 sequences in the viral vector with other viral sequences

Genetic material may be exchanged because of interaction of viral sequences present in the construct (CMV and SV40 sequences) with other wildtype viruses present in the patient or non-target individual.

Hazards related to insertional mutagenesis

A theoretical risk of AAV infection is the risk of insertional mutagenesis caused by non-site-specific integration of the AAV genome into the host cell genome of infected cells.

Hazards related to vertical transmission

Germline transmission may occur if a productive infection and stable integration of the viral vector occurs in germ cells.

Potential hazards to animal health

Unintentional exposure of animals to the GMO may result in expression of the normal human SMN protein and induction of an immune response against the Zolgensma capsid proteins.

Evaluation of potential consequences

The consequences of the identified potential adverse effects are evaluated, in case they occur. The magnitude of each effect is assigned a relative weighting ranging from high, moderate, low to negligible.

Consequences of shedding of vector particles

Unintentional exposure of non-target individuals to the GMO may result in expression of the normal human SMN protein and induction of an immune response against the Zolgensma capsid proteins. The SMN protein will be constitutively expressed. This protein is of human origin and thus no immunogenic effect is expected. In the Zolgensma-CL-101 study, no immune response against the transgene was observed in the treated patients. According to the applicant, there are no known toxicities related to the expressed SMN protein. The *SMN1* gene is the primary producer of SMN protein, a ubiquitously expressed protein that is essential in all tissues and is not associated with toxicity when overexpressed. While the protein product (SMN) from a single functional *SMN* gene is sufficient to prevent development of SMA, many individuals (13%) express 3 copies of *SMN1*, and almost 1% express 4 copies of *SMN1*, with no observable or known toxicity. Given the high prevalence of 4 copies of *SMN1* in the general population (without related pathology), it is clear that there is no significant toxicity related to significant overexpression of SMN in humans. The GMO is replication deficient and AAV is not pathogenic. An immune response against AAV9 capsid proteins will be asymptomatic, in the same way that a naturally occurring infection with AAV induces an asymptomatic immune reaction. The GMO has a wide biodistribution and it is plausible that shedding will occur. According to the applicant, the consequences of shedding of vector particles are low.

Consequences related to complementation, recombination and rcAAV formation

Mechanisms that might lead to increased pathogenicity rely on the generation of a novel virus through recombination of viral vector sequences with wildtype AAV sequences.

In a worst-case scenario, a replication-competent AAV expressing the human SMN gene would efficiently spread from patients to the environment. The host-range of such a virus would include humans and other primates. It is not expected that expression of *SMN* would positively affect the viral biology of the

vector with regard to replication and pathogenicity. Furthermore, new replication competent viral particles that have acquired the *rep* and *cap* genes because of recombination will most likely be similar to wildtype AAV due to the limited packaging capacity of AAV. Wildtype AAV infections are common and not associated with disease. According to the applicant, the consequences of recombination are negligible.

Consequences related to recombination of the CMV and SV40 sequences in the viral vector with other viral sequences

Homologous recombination between the GMO and wildtype CMV or wildtype SV40 will only result in reciprocal exchange. No consequences are associated with such a recombination because the properties of CMV or SV40 will not change due to recombination with the CMV or SV40 sequence from the GMO.

Consequences related to insertional mutagenesis

Preclinical data indicate that in most cases, DNA delivered by recombinant AAV vectors predominantly persists as extrachromosomal elements (episomes) rather than integrating into host cell genomes. The potential risk of incorporation of the viral genome into the patient chromosomal DNA is thought to be significantly reduced, as recombinant AAV vectors devoid of the *rep* gene are incapabe of site-specific integration into the AAVS1 site. However, the possibility of rare integration events may exist. According to the applicant, the consequences related to insertional mutagenesis are negligible.

Consequences of vertical transmission

Vertical transmission of AAV is not observed in (pre)clinical studies but cannot be completely excluded. However, Zolgensma is reasonably expected to be used in infants only. Overall, the magnitude of consequences associated with vertical transmission has been rated as negligible.

Consequences to animal health

The host-range of AAV9 includes humans and other primates. Unintentional exposure of animals to the GMO may result in expression of the normal human SMN protein and induction of an immune response against the Zolgensma capsid proteins. The SMN protein will be constitutively expressed. This protein is of human origin and an immunogenic effect might be expected in primates. According to the applicant, there are no known toxicities related to the expressed SMN protein. The GMO is replication deficient and AAV is not pathogenic. An immune response against AAV9 capsid proteins will be asymptomatic, in the same way that a naturally occurring infection with AAV induces an asymptomatic immune reaction. The GMO has a wide biodistribution and it is plausible that shedding will occur. The consequences of shedding of vector particles are rated as low.

2.3.4.2. Evaluation of likelihood

The evaluation of likelihood considers the probability that previously identified harmful effects occur.

Likelihood that consequences occur due to shedding of viral particles

After administration the GMO will be able to enter the environment for a limited time by shedding. From a previous clinical trial with Zolgensma, it appears that shedding can occur in urine, saliva and stool. Levels representing 0.1 – 0.01% of the initial dose into the patient are found in urine and saliva at 1 day post-dosing, after which levels of Zolgensma shed into these matrices reach near the limit of quantitation of the assay. In stool, concentrations were declining approximately 4 logs (10,000-fold) over 30 days post infusion. Overall, Zolgensma was primarily cleared from the body in stool and by day 60 post infusion was below the limit of quantitation in stool. Because the amount of vector particles in non-target individuals will be many times lower than in patients, the chance of negative effects is reduced compared to the situation in patients. The likelihood of further spreading of the GMO from these non-target

individuals is negligible since the GMO is replication deficient. Additionally, the presence of viral vector DNA does not necessarily mean that infectious particles are present. Preclinical studies with recombinant AAV show that urine contains no infectious AAV particles and that infectious vector particles are limited to the plasma and removed from the circulation within 48 to 72 hours after administration.

Also, replication deficient viral particles with non-vector related sequences can be temporarily excreted in the environment. When non-target individuals are exposed to AAV virus particles with non-vector related sequences, such as the *rep* gene, kanamycin DNA or host cell DNA, no adverse effects are to be expected. Over 90% of the adult human population is seropositive for AAV. As a result, the immune system recognizes and eliminates AAV particles. In the unlikely event that a possible infection of non-target individuals occurs, this effect will extinguish due to the replication defective nature of the viral vector.

The likelihood that shedding of viral particles occurs is high but the consequences are limited.

Likelihood that consequences occur due to complementation, recombination and rcAAV formation

During production, recombination of the viral vector with rep and cap sequences can result in the generation of replication competent AAV. In addition, there is a theoretical chance that in the patient rcAAV may arise due to recombination. Recombination between the vector and wildtype AAV will most likely result in exchange of homologous sequences such as the ITRs. This allows the *rep* and *cap* genes of the wildtype AAV to be exchanged with the SMN expression cassette of the GMO. The resulting virus contains the SMN expression cassette but will still be replication deficient due to the absence of the *rep* and *cap* genes. Furthermore, AAV has a limited packaging capacity, making it unlikely that an rcAAV vector will contain both the *rep* and *cap* genes and the transgene expression cassette.

Zolgensma is tested for replication competent AAV (rcAAV). The method has been validated and is being performed under GMP conditions. The proposed commercial acceptance criterion of this assay is a negative result. Analytical data from eight Zolgensma FP batches that were tested were all negative.

The provided data are insufficient to conclude that the rcAAV assay is valid for testing the absence of rcAAV in a batch of Zolgensma. Furthermore, the applicant indicates that during validation of the assay, a default value was tested based on the amount and concentration of the material that AveXis was able to provide and that this level was continued to be used throughout validation and testing with the rcAAV assay. However, the amount of Zolgensma particles tested in the rcAAV assay is still unknown, since it is not mentioned how much material (e.g. how many µl) is used in the assay.

However, the pSMN plasmid does not contain any elements of AAV replication or capsid proteins and the pAAV2/9 plasmid does not contain any sequences related to the AAV inverted terminal repeats, minimizing the likelihood of recombination and formation of rcAAV.

After administration of the GMO, complementation of *rep* and *cap* genes can occur in the presence of wildtype AAV and replication of the vector may occur. The presence (co-infection of the same cell) of a helper virus, for example adenovirus or herpesvirus, is required for this. Complementation by wildtype AAV and a helper virus can result in the generation of new vector particles, which can subsequently infect another cell. In this newly infected cell, replication will only occur if the same conditions are met. This temporary replication will not occur in the absence of the helper virus and wildtype AAV and, as a result, any spreading of the virus will eventually be extinguished.

In conclusion, the likelihood that infectious rcAAV is present in Zolgensma and is able to spread to non-target individuals is very low according to the applicant.

Likelihood of recombination of the CMV and SV40 sequences in the viral vector with other viral sequences

Theoretically, it is possible that a recombinant virus is produced by recombination of the CMV or SV40 sequence present in the AAV vector with wildtype CMV or wildtype SV40, respectively. According to the applicant, natural exchange of genetic material between the AAV vector and the donor organisms (CMV, SV40) is not known.

Likelihood that consequences occur due to insertional mutagenesis

As far as known, no events of insertional mutagenesis were observed in clinical trials involving recombinant AAV vectors. According to the applicant, the likelihood of insertional mutagenesis is negligible.

Likelihood of vertical transmission

Zolgensma is reasonably expected to be for used in infants only (SMA type 1 patients weighing between 2.6 kg to 21 kg). According to the applicant, Zolgensma does not integrate into the patient genome, germline cells are not impacted and the likelihood for germline transmission is negligible.

Likelihood of consequences to animal health

The host-range of AAV9 includes humans and other primates. The product is intended as a medicinal product for humans only and to be administered in a PICU patient room or other appropriate setting (e.g. interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management. After administration, Zolgensma can be shed, mostly via feces. The concentrations of the GMO that will end up in the environment will be many times lower than the administered dose. According to the applicant, exposure of animals is not expected. As such, the likelihood of consequences to animal health is negligible.

2.3.4.3. Estimation of the risk

Risk of shedding of viral particles

The viral vector is replication deficient. After administration, the GMO will be able to enter the environment for a limited time by shedding. AAV infections are common and not associated with disease. The presence of viral vector DNA does not necessarily mean that infectious particles are present. Both for the AAV vector and for the SMN sequence cloned herein, it has been concluded that the risks for humans and the environment are negligible.

Risk of complementation, recombination and rcAAV formation

During production, recombination of the viral vector with rep and cap sequences can result in the generation of replication competent AAV. As part of quality control, Zolgensma is tested for rcAAV. Analytical data from eight Zolgensma FP batches that were tested were all negative.

However, the provided data are insufficient to conclude that the rcAAV assay is valid for testing the absence of rcAAV in a batch of Zolgensma and the amount of Zolgensma particles tested in the rcAAV assay is still unknown. Although the pSMN plasmid does not contain any elements of AAV replication or capsid proteins and the pAAV2/9 plasmid does not contain any sequences related to the AAV inverted terminal repeats, thereby minimizing the likelihood of recombination, it cannot fully be excluded that there is rcAAV present in a batch of Zolgensma.

Replication of AAV requires a helper virus and, as a result, any spreading of rcAAV will be limited. Additionally, recombinant AAV vectors have low infectivity and require high virus titers for efficient transduction of cells. Thus, in the unlikely event that rcAAV particles are shed from patients, these rcAAV particles hardly have the capacity to cause significant infections. Importantly, new replication competent viral particles that have acquired the rep and cap genes because of recombination will most likely be

similar to wildtype AAV due to the limited packaging capacity of AAV. Wildtype AAV infections are common and not associated with disease.

In the patient, there is a possibility that new viral particles are formed as a result of complementation or recombination of the GMO with AAV *rep* and *cap* genes. To this end, it is necessary that the GMO, wildtype AAV and a helper virus are present in the same cell. No harmful effects are associated with the development of these new viral particles. New viral particles formed by complementation are identical to the GMO and will still be replication deficient due to the absence of the *rep* and *cap* genes. New replication competent viral particles that possess the *rep* and *cap* genes because of recombination will most likely be similar to wildtype AAV due to the limited packaging capacity of AAV. The likelihood of the formation of these new viral particles is very unlikely because the GMO, wildtype AAV and a helper virus must be present in the same cell at the same time.

Overall, the risk of complementation, recombination and rcAAV formation is regarded as negligible

Risk of recombination of the CMV and SV40 sequences in the viral vector with other viral sequences

Homologous recombination between the GMO and wildtype CMV or wildtype SV40 cannot be excluded but will only result in reciprocal exchange. The risk of recombination of the CMV or SV40 sequences with CMV or SV40, respectively, in the patient or non-target individual is negligible.

Risk of insertional mutagenesis

A theoretical risk of AAV infection is the risk of insertional mutagenesis caused by non-site-specific integration of the AAV genome into the host cell genome of infected cells. As far as known, no events of insertional mutagenesis were observed in clinical trials involving recombinant AAV vectors. According to the applicant, the risk of insertional mutagenesis is negligible.

Risk of vertical transmission

Vertical transmission of AAV is not observed in (pre)clinical studies but cannot be completely excluded. Human data on use of Zolgensma during pregnancy or lactation are not available and animal fertility or reproduction studies have not been performed. However, Zolgensma is reasonably expected to be for used in infants only (SMA type 1 patients weighing between 2.6 kg to 21 kg). Overall, the risk of vertical transmission can be considered as negligible.

Risk of effects on animal health

The viral vector is replication deficient and does not replicate in the absence of a helper virus. After administration, the GMO will be able to enter the environment for a limited time by shedding. The host-range of AAV9 includes humans and other primates. According to the applicant, exposure of animals is not expected. As such, the risk of effects on animal health is negligible.

Risk estimation

In conclusion, the risk for the environment that is related to the use of Zolgensma is considered negligible.

2.3.4.4. Risk management strategies

Even though the overall risk of transmission or adverse events associated with Zolgensma is negligible, a series of measures have been taken by the applicant to minimize the likelihood of spread in the environment or to non-target individuals. These are related to production, preparation and administration of the GMO, transport and waste treatment.

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

Because the environmental risks of Zolgensma are negligible, the inclusion of additional risk management strategies for reasons of environmental safety and safety of non-target individuals is not necessary.

2.3.4.5. Determination of the overall risk

The overall risk for the environment posed by the GMO is negligible.

2.3.5. Discussion on the non-clinical aspects

Pharmacology

The applicant suggests that the clinical benefit is more likely related to the progressive loss of motor neurons than a change in transduction efficiencies as a result of age. While it is true that the AAV vector can only transduce neurons that are still viable, it has also been demonstrated that with increasing postnatal age, the percentage of transduced glial cells is increasing, while the number of transduced neurons is decreasing depending on the CNS structure investigated.

Age may influence the transduction efficiency upon i.v. administration in a healthy animal. Data on transduction efficiency (the percentages of transduced neurons) in NHPs treated at postnatal day 30 and 90 was not present, consequently limiting the conclusions on the influence of age on transduction of neurons. Although AAV9 vector is able to reach the target tissue, there is no convincing quantitative data available from NHP that would support a sufficient transduction efficiency of motor neurons after intravenous administration of Zolgensma to SMA patients at an age of > 6 months of age.

Pharmacokinetics

Because of the discrepancy between the human patient biodistribution data at single cell level and the murine biodistribution data at whole tissue level, the Applicant was asked to provide an overview of all available human whole tissue and single cell biodistribution data in comparison to whole tissue murine biodistribution data (from the GLP 12-week studies) to determine similarities and differences in transducability per tissue between mouse and human and discuss the expected murine biodistribution at single cell level based on these human data. Murine biodistribution data from the 12 and 24 weeks study was submitted. Biodistribution data from two patients (study CL303and CL-302) that have been treated with Zolgensma at different ages (62 days versus 5 months of age, respectively) was also presented. In mouse, the amount of vg/µg DNA seems to slightly decrease over time. It is not clear whether this is due to a biological process or whether this variation is inherent to the assay. When comparing 12-weeks mouse to 24-weeks human data, it is observed that in brains, humans have slightly higher levels of vg/µg DNA, whereas levels in Lumbar Spinal Cord are more similar between mouse and human. It is reassuring that in brain and spinal cord at least similar levels as in mice are reached in human. In the periphery, organs such as lung, heart and quadriceps muscle do show similar levels of vector genomes per µg DNA for mice and human. However, liver and especially spleen seem much more efficiently transduced in human when compared to mice.

When comparing the two patients from study CL-303and study CL-302, it is observed that overall, the patient from study CL-302seems to be more efficiently transduced, often ± >1,5 times higher.

Unfortunately, no data from the brain for patient from study CL-302 were presented by the applicant; however, these data were present in the clinical dossier. These data suggest that Zolgensma distribution to brains is approximately a factor 10 higher the patient in CL-302 as compared to the patient from CL-303. Of note, it is not clear whether the data taken from the clinical dossier are collected in the same manner as the data presented in the applicant's position for the non-clinical part. Surprisingly the pancreas seems more efficiently transduced in the patient from study CL-302, whereas heart, spleen and inguinal lymph node seem more efficiently transduced in the patient from study CL-303. Apparently, interpatient differences do exist.

Overall, the above presented data at least provides an indication that mice and man are similarly transduced, with some differences in peripheral tissues. Furthermore, changes between individual patients are present. Whether this is due to the age at time of transduction or whether this is influenced by other factors is not known but might be of interest for future research in this field.

Although prednisolone is a prescribed co-medication, pharmacokinetic drug interaction studies are not submitted. However, it may influence persistence and clearance of Zolgensma. The applicant elaborated on the reasons for the prednisolone treatment of the treated patients and indicated that a prophylactic or therapeutic use of prednisolone has not been modelled prospectively in preclinical studies, which is considered obvious. Unfortunately, a non-clinical study to the influence on and potential refinement of the prophylactic or therapeutic use of immunomodulatory agents for this *in vivo* gene therapy are not planned.

Toxicology

The relationship between the occurrence of cardiovascular toxicity and the use of the specific FVB mouse strain as well as the age of the animals at start of dosing are not clear. Insight in mechanisms underlying atrial thrombosis are limited. The occurrence of cardiovascular toxicity in the clinic should be addressed in the clinic as extrapolation from non-clinical data is not possible.

Differences in study outcomes (reversibility of the lesions and the occurrence of clinical signs and mortality) between both pivotal 12-week studies are present. The applicant considered distinct pathological terminology the reason for potential differences in clinical and pathological observations between study no. 20122446 and no. 8384031, explaining part of the differences. Toxicity in both studies occurred in the same organs (primarily liver and heart) and comparable lesions were found (e.g. atrial thrombosis). Differences in the severity of these lesions may be assigned to distinct pathological terminology, although liver findings were not reversible by week 12 in study no. 8384031, while recovery was found in study no. 20122446.

However, mortality is not regarded related to terminology. It is striking that none of the animals in the mid-dose group in study no. 20122446 (i.e. $2.37x10^{14}$ vg/kg) died prematurely, while considerable mortality was present in the comparable mid-dose group in study no. 8384031 (i.e. $2.4x10^{14}$ vg/kg: 6 animals died due to product-related toxicity). In addition, already in the low-dose group in the latter study (i.e. $1.5x10^{14}$ vg/kg) 3 animals died prematurely, although relation to the procedure or test article is not clear. Thus, although within study no. 8384031 a mortality dose-response was present, this dose-response is not fully seen when the results of both studies are taken together. The reason for the difference in mortality between both studies could have been incorrect dosing or differences in batch potency could have played a role but is not clarified. Because histopathology was described in more detail and peer-reviewed in study no. 8384031, results from this study are considered most reliable among the GLP studies in mice using the IV route of administration.

The applicant argued in their response to the second-round questions that the product-related cardiovascular and liver findings are most likely the result of AAV platform effects and not specific for

the product or the transgene. Although literature (e.g. Hinderer et al., 2018) confirms the presence of platform effects, product-specific toxicity due to immune responses against the transgene or high/broad transgene expression in animals cannot be excluded(as also e.g. stated in Domenger and Grimm, Human Molecular Genetics, vol. 28, 2019 with specific attention for the second paragraph on page 3 and corresponding references). The promotor used in the AVXS101 vector could play a role in this very high transgene expression in certain tissues, resulting in stress-related cell death.

The applicant mentions that no abnormal cardiovascular observations have been made in patients (except for transient increases in troponin-I). It is likely that the concurrent use of prednisolone has a protective effect on both liver and heart, thereby preventing life-threatening conditions (inflammation) as seen in mice. Nevertheless, the applicant is aware of the need to monitor cardiovascular and liver parameters in patients, which is encouraged.

Although the Applicant has provided some information of the development of ventricle lesions over time. No discussion related to the atrium lesions (ultimately leading to thrombosis), liver lesions or the other pathology found in the tissues was provided. Moreover, the Applicant was asked elaborate on the (temporary) haematological changes and there clinical relevance. The Applicant should also discuss the most likely sequence of the different findings (i.e. clinical events, haematological findings, pathological lesions, reversion of lesions, mortality), starting with the transduction of specific tissues with the vector, leading to the adverse events found in the studies (i.e. which effects first, which effects as a result of that, etc.). The applicant only elaborated at high level on the development of pathological lesions, most likely because these lesions are already present at 3 weeks and no mechanistic toxicity studies have been performed. In addition, no attempts to explain (product-related) effects on haematological parameters such as thrombocytopenia were made. As the symptoms are manageable in the clinic, the lack of mechanistic understanding can be agreed.

During the third round, the applicant announced that it appeared that two more non-clinical studies were conducted that appeared relevant for but were not submitted with the Zolgensma application currently under review at the EMA. This concerned 1) a (final) report for an early GLP toxicology study in the mouse initiated in February 2017 and with final draft report issued in August 2017 and 2) final contributor histopathology report (dated May 6, 2019) as well as the remainder of the study report from a non-GLP intrathecal administration cynomolgus monkey biodistribution study with Zolgensma with or without two types of intrathecal contrast agent. Approximately one week later, the applicant shared an high level assessment report with the authorities to provide information on new preclinical safety findings from an exploratory biodistribution and safety study in cynomolgus monkeys upon IT administration of the product. The study identified the cervical, thoracic, lumbar, and sacral dorsal root ganglia (DRG) as a new target organ of toxicity for Zolgensma following intrathecal dose administration. A summary from the applicant is provided below:

- *Cynomolgus monkeys negative for AAV9 antibodies were administered intrathecal (IT), Zolgensma in the presence or absence and euthanized 2 weeks post-injection. All animals survived the 2-week observation period with no clinical evidence of neuronal toxicity.*
- *In the dorsal root ganglia (DRG), minimal to marked mononuclear cell inflammation was observed, with neuronal satellitosis, neuronal necrosis, or complete neuronal loss with rare mineralisation at histopathology severity grades of mild, moderate or marked (grades 2-4).*
- *The DRG was not identified as a target organ of toxicity in previous Zolgensma studies conducted in mice (ICV route of administration) or cynomolgus monkeys (IV or IT routes of administration). However, similar findings have been reported after administration of adeno-associated virus serotype 9 (AAV9) vectors in monkeys and minipigs (AveXis Study RPT-1183; Hinderer et al., 2018; Hordeaux et al., 2018).*

- *The Zolgensma batch used on this study did not meet GMP specifications, and the study had no concurrent vehicle control group.*

In conclusion, given that these findings were not previously observed with Zolgensma administration, they represent a new, adverse, potentially serious, and unexpected safety finding for Zolgensma.

It appears that in the NHP IT study submitted with the IV application (no. CSF-AAV9-SMN-NHP 001), in which vacuolisation in DRG neurons was observed in 1/3 animals (regarded background finding, by then), animals were dosed 2x lower as in the newly presented study data (no. ITFS-101). Furthermore, toxicity findings in DRG neurons were also observed in a study with AAV9 administered IV to NHP (and piglets) as published by Hinderer and colleagues (2018) in which the animals were dosed 2×10^{14} Vg/Kg, apparently 2x higher that the proposed clinical dose for the current IV application. In a study published by Hordeaux and colleagues (2018), DRG toxicity was also observed in NHP after an ICM administered dose of $1x10^{13}$ vg AAV9-IDUA. In their notification, the company referred to an AVEXIS study in which, with an IT dose of another AAV9 vector, also inflammation and degeneration of the DRG neurons was observed.

Quick comparison of all these data may suggest that the dose level could be influencing the occurrence or the revelation of the effect on DRG neurons in the spinal cord. The applicant was asked to elaborate on the consequence of this finding for the current intravenous application. In the discussion the applicant was asked to consider the differences in dose levels used in the various studies, to compare the findings in IT and IV studies with AAV9 (both private and literature data) and touch upon mechanism of action, use of corticosteroids and translatability.

Overall, the applicant has made assumable that DRG toxicity that is observed within 2 weeks up to a few months post administration is possibly related to both innate and adaptive immune responses upon AAV9-mediated *in vivo* transduction. DRG findings in ITFS-101 were observed in Zolgensma administered young adults that were analysed two weeks post injection when the immune response may have been maximal. Animals in study CSF-AAV9-SMN-NHP-001 were given an approximately 2fold higher dose at younger age, but only analysed 18 months post injection, and not specifically for pathological DRG toxicity. Absence of DRG toxicity findings in this study may have several reasons. It could be the case that DRG toxicity was missed as DRG was not a target organ for microscopically histopathological analysis, or that younger animals are less susceptible or that DRG toxicity was reversed after 18 months and therefore not visible anymore. In NHP, DRG toxicity seems not correlating with neurological symptoms, in contradiction to piglets, which showed effects of proprioceptive deficits and ataxia that are regarded species-specific. In several studies from the applicant or studies published in literature it has been shown that in NHP immunosuppression attenuates effects related to innate and adaptive immune responses in animals (and human). Attenuation of DRG toxicity upon immune suppression was not so clear in animals after ICM (intra cisterna magna) administration of AAV9-IDUA or AAV9-IDS to rhesus macaque. However, it may not exclude that it could attenuate DRG toxicity in human, thereby further downgrading the risk for DRG toxicity in patients treated in the clinic with Zolgensma.

Severity of DRG findings may be dose-dependent and it is suggested that the local vector concentration for DRG neurons is relatively high upon IT administration as compared to IV administration. The applicant suggests that this may differ with a factor 10 and that this factor may be applied when extrapolating IT data to IV application. In addition, also not so clearly stated in the answer of the applicant, a factor 10 could be applied between NHP and human with regard to extrapolation of IT data as cynomolgus monkey are thought to have only 10% of the humans CSF volume.

The applicant predicts that IV DRG findings would remain sub-clinical through a dose range up to ~3-5e15 vg administered intravenously. These doses are approximately 3-5-fold the recommended clinical

dose of 1.1e14 vg/kg and suggests the risk of clinically relevant DRG findings is very low. It is not clear how the applicant comes to this safe dose. It is likely that this dose was determined on the absence of symptomatic DRG findings in clinical studies administering 2.4e14 vg Zolgensma, corresponding to 2.3e13 vg/kg, co-administered with immunosuppressive medication. When applying the proposed factor 10, a dose of 2.8e14 vg/kg IV in combination with immunosuppression would not likely to result in symptomatic DRG toxicity. But this dose is slightly lower than the safe dose as proposed by the applicant. Whether DRG toxicity on the pathological level would occur in human remains unclear. When applying the factor 10 to the NHP study ITFS-101 and extrapolate on a bodyweight basis, the ITFS-101 study would suggest that a dose of 1.08e14 vg/kg IV (without immunosuppression) would result in DRG findings such as minimal to marked mononuclear cell infiltration and neuronal necrosis in rare cases. When taking into consideration a factor 10 scaling based on the CSF volume difference between monkeys and human, it could be expected that a dose of 1.08e15 vg/kg IV to human in absence of immune suppressive medication would result in pathological DRG toxicity. However, DRG microscopic findings would not likely have symptomatic correlates.

In any case, it is regarded really valuable that monitoring for symptoms of DRG toxicity is proposed by the applicant upon IV administration of Zolgensma.

The Applicant was asked to submit and discuss findings in the reports of the two studies that were identified lately not to be communicated yet with the authorities, i.e. 1) an early GLP toxicology study in the mouse initiated in February 2017 with final draft report issued in August 2017, and 2) final contributor histopathology report (dated May 6, 2019) as well as the remainder of the study report from a non-GLP intrathecal administration cynomolgus monkey biodistribution study with Zolgensma with or without two types of intrathecal contrast agent.
The Applicant has provided both reports:

1) A 12-week GLP-compliant toxicity study was performed with a research development batch (produced in between process A and process B) to assess the potential toxicity of Zolgensma in the CNS. Mice were IV administered a single dose of $4.63x10^{13}$ vg/kg or $3.58x10^{14}$ vg/kg Zolgensma at PND1. This is approximately 0.4- and 3.3-fold the intended clinical dose of $1.1x10^{14}$ vg/kg. AAV genomic titer was determined with the validated ddPCR method. According to the Applicant, the batch contained 44% empty capsid and was therefore not representative of test material intended for use in the study.
   The current data raise considerable safety concerns related to Zolgensma at high dose (i.e. mortality with considerable liver damage, atrial thrombosis, pulmonary oedema and coagulopathy), while e.g. mortality was not present in the 24-week study no. AD49TV.7A32.BTL (conducted with pre-process A batch). Most likely, the Applicant did not consider the test material suitable for the current study and decided to perform two additional studies with process B-material, which is endorsed. However, these two studies (no. 20122446 and 8384031) resulted in comparable clinical signs, pathology (in liver and heart) and mortality as the current study. This suggests that the NC safety issues are not solely related to specific batches and that Zolgensma administration carries intrinsic safety risks for liver and cardiovascular damage with coagulopathy when used in young animals, especially at high doses. Clinically, the Applicant is aware of these risks, which can be (partly) mitigated with immune suppression and proper monitoring.
2) A 2-week (non-GLP) toxicity study was performed with a batch rejected for clinical/commercial use to assess the safety of IT-delivered Zolgensma. Male cynomolgus macaques were IT administered (with Trendelenburg position) a single dose at the age of 1.4-2.9 years. Animals were euthanised 2 weeks post-injection.
   IT administration of AAV9-GFP or Zolgensma led to transduction of spinal motor neurons throughout the whole spinal cord. Zolgensma resulted in DRG inflammation and perivascular mononuclear cell infiltrates in epineurium and meninges of both brain and spinal cord (and in several peripheral

tissues). DRG inflammation was rated minimal (i.e. no neuronal necrosis) to severe (i.e. neuronal necrosis or complete loss). Incidence and severity of this inflammation were the highest in sacral DRG compared to DRG more close to the brain.

3) DRG inflammation consisted primarily of $CD3^+$ lymphocyte infiltration, although a role of other immune cells (e.g. innate cells) in the inflammation was also observed. The extent of lymphocyte infiltration was related to the amount of AAV (i.e. genomic material or capsid protein) present within the DRG.

   Taken together, the presence of cell infiltrates in various tissues may be related to the IT delivery method and/or could even be (partly) considered a background finding (but no saline-injected control group was present to confirm or disprove this). Nevertheless, specific lymphocyte infiltration and neuronal damage seen in the DRG are most likely Zolgensma-related and considered adverse. The clinical relevance of this finding and whether it is also observed after IV administration remains unclear.

The Applicant was also asked to provide any histo(patho)logy data from CNS and peripheral tissues available from IV NHP studies (for example, but may be not limited to, the study entitled "*Analysis of Vector Safety Following a Single Systemic Delivery of scAAV9.CB.SMN into Cynomolgus Macaques*"). The Applicant states that only one IV study with Zolgensma has been performed in NHP using a research-grade batch, for which the dose was considerably lower ($6.7x10^{13}$ vg/kg) compared to the clinical dose. In addition, the Applicant states that "*histopathology changes noted in the IV dosed NHP Zolgensma study at 6.7e13 vg/kg were reported to be minimal and similar to controls.*" Nevertheless, no histopathological data have been provided. Because this report may have been poorly documented, data from the IT NHP study (with DRG histopathology data) and from publications may be more informative. These data are provided and assessed in the discussion of the Applicant related to the comparison of findings in IT and IV studies with AAV9 (see above).

Finally, the Applicant was requested to provide an update of section 5.3 of the SmPC with provided murine and/or NHP information if relevant to the prescriber for the current IV application. The Applicant proposed to add a paragraph on the DRG findings (after IT administration). The wording of this paragraph required further adaptation, which is further assessed in the separate SmPC document.

Based on the DRG events in NHP after IT administration and the uncertainties related to the relevance of DRG events for IV administration of Zolgensma, additional monitoring of sensory motor neurons functionality in patients treated with Zolgensma is considered needed. This is further assessed in the clinical AR.

2.3.6. Conclusion on non-clinical aspects

The Zolgensma related and dose-dependent major finding in the heart of mice after IV administered of Zolgensma at neonatal stages includes atrial thrombosis which results in unscheduled mortality in a significant number of animals. Zolgensma related cardiovascular toxicity have not been observed to occur in clinical studies with Zolgensma manufactured using the commercial manufacturing process (Process B). DRG toxicity as observed upon IT administration of Zolgensma to cynomolgus monkey are not likely to be observed in human upon the current proposed dose regimen. However, it is regarded really valuable that monitoring for symptoms of DRG toxicity is proposed by the applicant upon IV administration of Zolgensma.

Zolgensma can be approved from non-clinical perspective.

The CHMP endorsed the CAT conclusions on the non-clinical aspects as described above.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

To date, the clinical development program for Zolgensma includes 9 studies, 7 interventional and 2 observational. Six studies were included in the dossier. As of the cut-off date of 31 Dec 2019, 133 patients have received Zolgensma in clinical studies, 101 have received Zolgensma intravenously (98 of these at the proposed therapeutic dose and 3 at a lower dose), and 32 patients have received Zolgensma intrathecally.

Note In study CL-101 the process A product was used. In the studies CL-303, CL-302 and CL-304 the process B product is used.

Table 3:Tabular overview of clinical studies (Cutoff date 31 DEC 2019)

Study ID	No. of study centres / locations	Design/ Study objective	Study Posology	Subjects by arm entered/ completed.	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
CL-101	single-centre (US)	Phase I, open label, dose escalating study, Dose-finding, safety and efficacy	Single IV infusion Cohort 1: 6.7E13 vg/kg Cohort 2: 2.0E14 vg/kg	Cohort 1: 3 planned/3 treated, Cohort 2: 12 planned/12 treated	2 years post-dose. First patient enrolled: 05 May 2014, last patient completed: 14 Dec 2017.	M: n=6, F: n=9. Median age = 4.1 months (min: 0.9, max: 7.9). Age at vector infusion: ≤9 months for the first 9 patients dosed and ≤ 6 months for the last 6 patients dosed.	Type 1 SMA, bi-allelic SMN1 mutations, 2 copies of SMN2. Confirmed onset of disease at birth to up 6 months of age.	Primary objective: Safety, Primary efficacy endpoint: survival (defined as time from birth date to death or permanent ventilation[a)]
LT-001	single-centre (US)	Long-term, observational, safety, follow up study of CL-101. LT follow up	dosed in CL-101	15 planned/ 13 enrolled	15 years	see CL-101	see CL-101	medical history, physical examinations, clinical laboratory evaluations,
CL-303	Multi-centre (US)	Phase III, open label, single arm study, Safety and Efficacy	Single IV infusion of 1.1E14 vg/kg (equivalent to therapeutic dose cohort 2 CL-101)	20 planned/ 22 treated	Patients are followed up until 18 months of age.	M: n=10, F: n=12. Median age = 3.5 (min: 0.5, max: 5.9) months. Age at vector infusion: < 6 months of age.	Type 1 SMA, bi-allelic SMN1 mutations, 2 copies of SMN2 (ITT population), not excluding patients with 1 SMN2 copy or the SMN2 gene modifier c.895G>C.	co-primary endpoints: - survival at 14 months of age and - the proportion of patients who achieved functional independent sitting for ≥30 seconds at the 18 months of age study visit.

CL-102	Multi-centre (US)	Phase I, open label, dose escalating study, Dose finding for alternative RoA, safety and efficacy	Single IT infusion Cohort 1: 6.0E13 vg (dose A) Cohort 2: 1.2E14vg (dose B), Cohort 3: 2.4E14 vg	Cohort 1: 3 planned/3 treated, Cohort 2: 25 treated, Cohort 3: 4 treated	1-year post-dose	M: n=1, F: n=5. Median age = 19.6 months.	SMA, bi-allelic deletion of SMN1 and 3 copies of SMN2 without the genetic modifier (c.895G>C). Demonstrated the ability to sit unassisted for 10 or more seconds but have never been able to stand or walk at time of study entry. Onset of clinical signs and symptoms consistent with SMA at < 12 months of age.	For patients ≥ 6 months and < 24 months at the time of dosing: proportion of patients that achieve the ability to stand alone (Bayley Scales). For patients ≥ 24 months and <60 months at time of dosing: change from baseline in Hammersmith Functional Motor Scale
CL-304	Multi-centre (global)	Phase III, open label, single arm study, Safety and Efficacy	Single IV infusion of 1.1E14 vg/kg (equivalent to therapeutic dose cohort 2 CL-101)	Cohort 1 (2 SMN2 copies: 15 planned/14 treated. Cohort 2 (3 copies of SMN2): 12 planned/15 treated. Cohort 3 (4 copies of SMN2): cohort omitted. 1 patient included	Cohort 1: FU up to 18 months of age. Cohort 2: FU up to 24 months of age.	Cohort 1: M: n=4, F: n=10. Age at treatment (median): 21 (min: 8, max: 34) days. Cohort 2: M: n=6, F: n=9. Age at treatment: median: 31 (min: 9, max: 43) days.	Pre-symptomatic patients expected to develop SMA, bi-allelic deletion of SMN1 and 2,3 or 4 copies of SMN2 without the genetic modifier (c.895G>C) (ITT population). Patient with genetic modifier of SMN1 point mutations are not excluded.	Cohort 1: proportion of patients achieving milestone of independent sitting for at least 30 seconds at any visit up to 18 months of age. Cohort 2: proportion of patients achieving the ability to stand without support for at least 3 seconds at any visit up to 24 months of age. Cohort 3: proportion of patients demonstrating the ability to achieve a scaled score on Bayley scales within 1.5 SD of chronological development reference standard as assessed at 36 months of age.

CL-302	Multi-centre (EU)	Phase III, open label, single arm study, Safety and Efficacy	Single IV infusion of 1.1E14 vg/kg (equivalent to therapeutic dose cohort 2 CL-101)	30 planned/ 33 treated	Patients are followed up until 18 months of age.	M: n=14, F: n=19. Age at treatment (median): 4.1 (min: 1.8, max: 6.0) months.	Type 1 SMA, bi-allelic SMN1 mutations, 2 copies of SMN2 (ITT population), not excluding patients with 1 SMN2 copy or the SMN2 gene modifier c.895G>C.	the proportion of patients who achieved functional independent sitting for ⩾10 seconds (WHO criteria) at the 18 months of age study visit.

A: Survival, the primary efficacy endpoint, was defined as the time from birth date to either (a) death or (b) permanent ventilation, defined as requirement of invasive ventilation or ≥ 16 hours of respiratory assistance per day (including non-invasive ventilatory support) continuously for ≥ 14 days in the absence of an acute reversible illness, excluding perioperative ventilation. Permanent ventilation, so defined, was considered a surrogate for death.

2.4.2. Pharmacokinetics

Zolgensma is a viral gene therapy product to be administered intravenously. Conventional clinical PK studies are not possible for a viral gene therapy product. In humans, the presence of the viral vector in blood, shedding into excreta and the immunogenicity is investigated. Other PK studies for the viral vector are not feasible. The limited number of PK studies is acceptable for a viral vector.

The adeno associated virus serotype 9 (AAV9) viral vector carrying the SMN gene is designed for non-integration into the host genome and to reside as a deoxyribonucleic acid (DNA) episome in the nucleus of transduced cells. Recent literature suggests that SMN protein can be measured in plasma in excreted episomes. The Applicant will investigate biomarkers (SMN protein and neurofilament light and/or heavy chain) in intravenously treated patients at baseline and during LTFU, allowing for correlation of (pre-)treatment biomarkers and efficacy results.

Zolgensma is intended to be administered as a single IV infusion at a dose of 1.1 × 10E14 vector genome copies/kg in paediatric patients with SMA. Premedication with oral prednisolone should always be given 24 hours prior to infusion at a dose of 1 mg/kg/day and continued for at least 30 days followed by a tapering regimen. The prednisolone dose regime includes tapering off scheduled is described in the Product information.

To date, the clinical dossier for Zolgensma includes 6 studies in which the PK was investigated.

Analytical methods

The adaptive immunological responses to Zolgensma were monitored in study CL-101 using four different analytical techniques: ELISA for anti-AAV9 immunoglobulin G (IgG) antibodies, ELISpot for T-cell-mediated immunity to AAV9, ELISA for anti-SMN antibodies and ELISpot for T-cell-mediated immunity to hSMN. Furthermore, droplet digital polymerase chain reaction (ddPCR) was used to measure shed virus.

The analytical methods seem to be sufficiently validated to accurately determine the immunological response in patients after Zolgensma administration and to determine the shed virus. The ddPCR technique was not able to discriminate between empty viral capsules and intact Zolgensma containing the SMN1 gene DNA sequence. The anti-drug antibody (ADA) assays were not a quantitative assay.

Absorption

During the conduct of the studies, the Applicant changed the method used to determine the number of vector genomes in the administered dose and therefore it is difficult to compare the dosing between the studies. The highest dose used in the clinical trials was 1.1×10^{14} vector genome copies/kg using ddPCR. In study CL-101 also a lower dose was used in 3 patients.

Conventional clinical pharmacokinetic studies are not possible for a viral gene therapy product. Therefore, no PK parameters (e.g. AUC and C_{max}) are available. Absorption is 100% following intravenous administration. AVXS 101 is expected to behave as other AAV9s after intravenous administration. Therefore, data on biodistribution and shedding of other AAV9 could be used to support the biodistribution and shedding of Zolgensma. However, there is currently no literature on the biodistribution and shedding of AAV9 other than Zolgensma in humans and therefore no comparison can be made with other AAV9 viral vectors.

Distribution

The biodistribution in humans is based on information on the biodistribution in non-human primates. In non-human primates, long-term transgene expression was detected in most tissues, including liver, CNS,

heart, triceps, diaphragm, and quadriceps except for intestines which had little or no expression. This indicates that the viral vector is able to distribute from the systemic circulation into the tissues and transfect these tissues.

Human biodistribution data was submitted for two deceased patients (from study CL-303and study CL-302) and indicated that intravenously administered Zolgensma at a dose of 1.1×10^{14} vg/kg crosses the blood brain barrier in an extent sufficient for transduction of the CNS cells from brain to lumbar/sacral spinal cord. Furthermore, these biodistribution data indicate that intravenously administered Zolgensma at a dose of 1.1×10^{14} vg/kg effectively transduces cells throughout the body with detection of Zolgensma vector genomes in the CNS, all investigated peripheral organs and (skeletal) muscle. It also confirms the liver and heart being identified off-target organs of Zolgensma.

Excretion

Clearance of Zolgensma is primarily via the faeces and the majority of the dose is cleared within 30 days of dose administration. The risks for adverse effects in healthy humans after shedding is considered low.

Immunogenicity

An immunological response, as expected, was observed against AAV9 after intravenous administration of Zolgensma. The immunological response is expected to be transient but based on the long-term data derived from CL-101 and LT-001 seems to be sustained. No immunological response was observed against hSMN.

2.4.3. Pharmacodynamics

Zolgensma is designed to increase the expression levels of the SMN protein in the motor neurons. Proof of the mechanism of action mainly comes from the non-clinical studies.

No designated PD studies were carried out for this gene therapy product.

There are no validated pharmacodynamic markers of SMA drug response in the published literature.

CMAP (compound muscle action potential) and MUNE (motor unit number estimation) are commonly used to study neuromuscular function in neuromuscular disorders. In a recently published natural history study of subjects with SMA, the effects over time on CMAP (ulnar) in SMA patients were characterized and compared to healthy controls[1]. CMAP amplitude and area were stable in healthy controls (amplitude between 5.5-6 mV). CMAP amplitude was never higher than 0.6 mV in infants with SMA type 1 and who were 6-months of age or older.

MUNE represents the estimated number of motor neurons or axons innervating the muscle or group of muscles being tested. In a study of 89 subjects with SMA (types 1, 2, and 3), denervation was assessed via MUNE[2]. From cross-sectional data, changes in MUNE over time were dependent on age, SMA type, and *SMN*2 copy number. SMA Type correlated with MUNE values and functional outcomes, with lower MUNE values and worse functional outcomes in SMA type 1.

CMAP and MUNE were measured as indirect PD markers in study CL-101.

Study CL-101 is a Phase 1, open-label, single-infusion, ascending-dose, single-centre study to evaluate the safety and efficacy of Zolgensma in up to 15 patients with Type 1 SMA (see also section2.5.3.1.). The CMAP size was found using supramaximal stimulation of the motor nerve to a defined muscle or muscle group. It was recorded using surface electrodes and was representative of the sum of the surface detected motor unit action potentials from muscles innervated by that nerve.

[1] Kolb et al, Annals of Neurology 2017.
[2] Swoboda et al, American Neurological Association 2005.

Results

Patients treated with the advised dose (Cohort 2) achieved sustained improvements in both peroneal CMAP amplitude (1.67 mV, 320% increase) and ulnar CMAP amplitude (0.87 mV, 125% increase) at 12-months post-dose. Seven of the 10 subjects with CMAP values at the Month 6 Study Visit or later had ulnar CMAP amplitudes >0.6 mV. Two subjects had ulnar CMAP >5 mV at the Month 6 Study Visit. For comparison, healthy children have a mean ulnar CMAP amplitude of 5.5-6 mV.

At 24 months post-dose, patients in Cohort 2 achieved sustained improvements in both peroneal CMAP amplitude (2.20 mV, 634.3% increase) and ulnar CMAP amplitude (0.84 mV, 141.7% increase).

At 12 months post-dose, subjects in the low dose group (Cohort 1) achieved a slight improvement in peroneal CMAP amplitude (0.10 mV, 58% increase) while ulnar CMAP amplitude decreased at the 12-month post-dose time point (-0.10 mV, -33% decrease). At 24 months post dose, subjects in Cohort 1 achieved a slight improvement in peroneal CMAP amplitude (0.20 mV, 116.7% increase), while ulnar CMAP amplitude decreased (-0.10 mV, -33%).

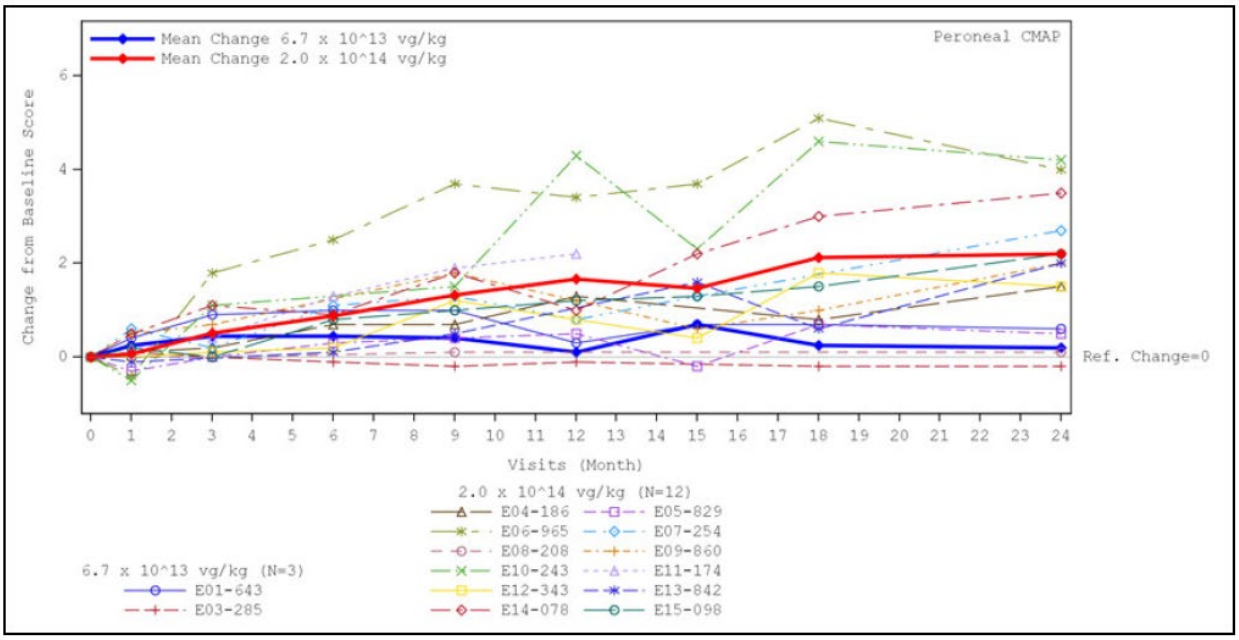


Figure 2:Mean Change for Ulnar CMAP [mV]

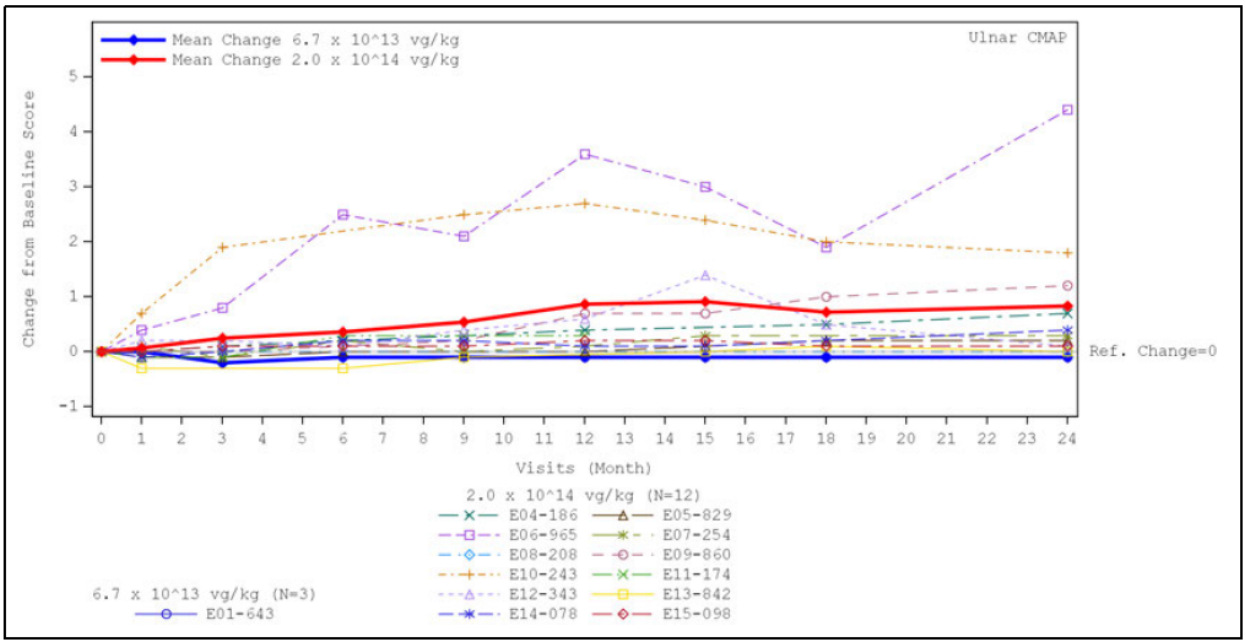


Figure 3:Mean Change for Ulnar CMAP [mV]

Patients in Cohort 2 achieved sustained improvements in MUNE (58% increase) at the 12 months post-dose time point. Subjects in Cohort 1 showed a 50% decline from baseline at the 12 months post-dose time point. At the 24 months post-dose time point patients in Cohort 2 achieved sustained improvements in MUNE (82% increase). Subjects in Cohort 1 showed a 34% decline from baseline in the 24 months post-dose time point.

The effects of Zolgensma produced according to process A on CMAP and MUNE are in general considered supportive of the overall clinical efficacy observed in the study.

Immune response and efficacy

A detailed immunogenicity report was submitted for study CL-101 (process A). The first patient treated with Zolgensma in Study CL-101 had increase in transaminases. In an attempt to dampen the host immune response to the AAV derived therapy, all subsequent patients were pre-treated with prednisolone prior to dosing.

T-cell mediated immunity and efficacy.

There was no apparent immune response against hSMN1. After treatment T-cell mediated immunity against AAV9 was present in all patients as measured by ELISpot.

In patients with the highest ELISpot values, similar responses in CHOP-INTEND score and motor milestone achievement (secondary endpoints, see efficacy section) were observed as compared to patients with lower ELISpot values.

This suggests that there are no relationships between baseline and post-baseline ELISpot and efficacy.

Humoral immune response and efficacy.

There was no apparent immune response against hSMN1.

All patients had increases in anti-AAV9 titres throughout the course of the study. These increases are considered an expected response to treatment with Zolgensma. Most patients (10) had increases in anti-AAV9 titres to > 1:50 at week 1, with even greater increases thereafter. Eleven patients had titres

greater than the limit of quantification (> 1:819200) at some point during the study. No patients returned to titres < 1:50 after dosing. All patients remained elevated (≥ 1:25600) at the last reported time point and 3 patients had anti-AAV9 titres greater than the limit of quantification at the last reported time point.

Of the 2 patients who had pre-dose anti-AAV9 antibody titres of 1:50 , increases in CHOP-INTEND scores in these patients were comparable to patients receiving the therapeutic dose and both patients achieved motor milestones of sitting alone ≥ 30 seconds, which surpasses or is equivalent to the milestones achieved of 6 of the other 10 patients in Cohort 2.

Eight patients out of 12 in Cohort 2 had anti-AAV9 titres that exceeded the higher limit of quantification. The development of anti-AAV9 antibodies had no apparent impact on efficacy. In patients who had anti-AAV9 titres that exceeded the limits of quantification, there were no apparent differences between patients who had larger anti-AAV9 titres and clinical outcome as measured by survival, achieving milestones or CHOP-INTEND. With the exception of patient E08, all patients from Cohort 2, achieved and maintained CHOP-INTEND motor function scores greater than 40. Seven of these patients had anti-AAV9 titres that exceeded the higher limit of quantification. Two patients in Cohort 2 achieved CHOP-INTEND scores above 60 points and both had anti-AAV9 titres that exceeded the level of quantification.

2.4.4. Discussion on clinical pharmacology

Pharmacokinetics

Generally, the pharmacokinetics of Zolgensma have been sufficiently evaluated.

Pharmacodynamics

The underlying cause of 5q SMA is a bi-allelic deletion/ point mutation of the *SMN1* gene, making patients dependent on the insufficient production of the SMN protein from the SMN2 gene. By replacing mutated SMN1 protein with fully functional SMN1 in motor neurons, Zolgensma is expected to contribute to motor neuron survival and extend survival in SMA patients. Proof for this mechanism of action is primarily obtained from non-clinical studies since it is not possible at the moment to measure SMN1 protein in treated patients. However, there are indications that SMN1 protein can be measured in plasma in excreted episomes. The Applicant will measure SMN levels in Study CL-305 to explore the relationship of SMN protein levels to onset and extent of clinical efficacy. In addition, the Applicant will add SMN protein level measurement to the LT-001 and LT-002 protocols to explore the relationship of SMN protein levels to durability of clinical efficacy.

There is also no validated pharmacodynamic marker for SMN1 protein replacement. Therefore, no dedicated PD studies were conducted. However, pharmacodynamic measures of muscle innervation were investigated in the dose-finding study CL-101, i.e. CMAP (compound motor action potential) and MUNE (motor unit number estimation). In the low dose cohort (cohort 1), CMAP did not improve consistently 12 and 24 months post-dose. In the high dose cohort (cohort 2), CMAP did improve both 12 and 24 months post-dose, above the level that would normally be expected from natural history.

In the NeuroNext study, CMAP was shown to positively correlate with motor functional ability. In addition, the patients with the highest increase from baseline in CMAP in study CL-101 (E06-965 and E10-243) were also the patients that reached the highest motor milestone and CHOP-INTEND score in study CL-101 (walking unassisted), see clinical section.

The genotype (i.e. number of *SMN2* copies) of a 5q SMA patient is roughly predictive for the phenotype and thus the progression of the disease. In the presence of a bi-allelic mutation/deletion of *SMN1*, having 2 *SMN2* copies is 97% predictive for developing SMA type 1[3]. With higher *SMN2* copy numbers, the

[3] Feldkotter et al, Am. J. Hum. Genet. 70:358–368, 2002

association with phenotype becomes more complex. Large studies (3459 cases) demonstrated that in patients with 3 *SMN2* copy numbers the three major phenotype categories are present: ~15% have a SMA Type 1 phenotype and never sit, ~55% have SMA Type 2 who sit but never stands/walks, and ~30% have SMA Type 3[4] (Calucho 2018). Per protocol, patients with 1 or 2 SMN2 copies could be included in study CL-303 and CL-302. The ITT population in studies CL-101, CL-303 and CL-302 consisted of patients with 2 copies of *SMN2.* A number of PCR-based copy number assays have been validated to quantify *SMN2* copy number in DNA samples from SMA patients. These methods are widely available in all EU member states. Available techniques can reliably determine *SMN2* copy number, especially in patients with up to and including 3 copies of *SMN2*[5][6].

No significant immune response was detected against SMN1, both at baseline and during the study.

All patients developed antibodies against the AAV9 capsid.

Subjects with anti-AAV9 titres > 1:50 were excluded from the study because of the possibility that high levels of anti-AAV9 antibodies in the subject prior to dosing might impair efficacy or result in adverse events. In study CL-101, one of sixteen screened patients were excluded due to a to high baseline AAV9 antibody titre. Two patients in CL-304 and 5 patients in CL-302 failed screening due to presence of AAV9 antibodies. Overall, 5.5% of patients screen failed due to antibody titer across the clinical program. This indicates that a AAV9 titre above 1:50 is not a major problem in clinical practice. This is supported by literature that reports low prevalence of AAV9 titres in children and young adults (6-10%). The available data do not suggest a major impact of AAV9 antibodies on safety and efficacy up to a titre level of 1:50. The upper border of the safe range remains undetermined. A warning in the SmPC is considered adequate to manage the possible risk of baseline AAV9 antibody titres.

None of the patients returned to AAV9 Ab titres <1:50 at the end of the study, posing a problem for re-treatment. For the moment, a re-treatment with Zolgensma should not be scheduled given the sustained anti-AAV9-antibodies after Zolgensma. This should also be reflected in the SmPC.

2.4.5. Conclusions on clinical pharmacology

No dedicated PK and PD studies were conducted for Zolgensma. This is acceptable for a gene therapy product. Bio-distribution in humans is largely based on data from non-human primates and data from the deceased patients.

Shedding of the virus occurs primarily via the faeces and the majority of the dose is cleared within 30 days of dose administration.

CMAP and MUNE were measured as pharmacodynamic markers in study CL-101. Increases in these parameters were shown after Zolgensma treatment. The increases exceed what can be expected from natural history.

No patients had baseline titres against SMN1 protein above the 1:50 limit, and there was no immune response against SMN1 after Zolgensma administration.

An immune response was mounted, as expected, against the AAV9 capsid. This immune response was dampened by the pre-treatment with prednisolone. There is no evidence that the formation of AAV9 antibodies prevent efficacy or poses a safety risk, nor is there evidence that baseline AAV9 seropositivity will occur frequently in clinical practice. The applicant included a warning in the SmPC that efficacy and safety is not established in patients with baseline AAV9 antibody titres above 1:50.

[4] Calucho et al, Neuromuscular Disorders 28: 208–215, 2018
[5] Gómez-Curet I et al, Neurogenetics 10(2):171-2, 2009
[6] Prior TW et al, Genet Med 13(7):686–694, 2011

The CHMP endorsed the CAT assessment regarding the conclusions on the Clinical pharmacology as described above.

2.5. Clinical efficacy

2.5.1. Dose finding

No specific dose response studies were conducted for Zolgensma. The justification of the Applicant of the therapeutic dose of 1.1E14 vg/kg (referred to as 2.0E14 vg/kg in study CL-101) is based on non-clinical studies and on study CL-101. The doses selected for study CL-101 were based on the observed safety and demonstrated efficacy in multiple animal models (see section0). Three dose levels were pre-planned (6.7E13 vg/kg, 2.0E14 vg/kg, and 3.3E14 vg/kg). The highest was never used as the second dose level exceeded expectations by motor milestone achievement never seen in the natural course of disease. This is also the dose selected for the pivotal study CL-303.

2.5.2. Main studies

The natural history studies PNCR and NeuroNext (see section below) provide the information of the natural course of the disease.

Study CL-303 evaluating process B manufactured product is considered the pivotal study for the assessment of efficacy in patients with 2 *SMN2* copies. The pivotal study for the assessment of efficacy, at the time of initial submission was study CL-101 in which the product was manufactured using process A. Because - based on Quality assessment - comparability of process A and B cannot be concluded CL-101 is now considered supportive. The process B product is investigated in study CL-302 and CL-304 also, which are still ongoing. Data from study CL-304 is used to support the use of Zolgensma in pre-symptomatic patients with 2 or 3 *SMN2* copies.

Study CL-303 is a Phase 3, open-label, single-arm, single-dose study of Zolgensma in patients with SMA Type 1 who were either symptomatic or pre-symptomatic with no functional SMN1 gene and 1 or 2 copies of *SMN2* and who are < 6 months (< 180 days) of age at the time of gene replacement therapy (Day 1). As of the 31st of December 2019 cutoff, the study has been completed. The final study report is awaited and will be provided in September 2020.

Since SMA patients have very limited motor skills, it is difficult to assess motor ability in these patients. The CHOP-INTEND (The Children's Hospital of Philadelphia Infant Test for Neuromuscular Disorders) score is a sensitive score for motor function ability designed and validated specifically for severely affected SMA patients. The patients are scored on 16 items (including joint flexion and extension, spontaneous movement and head control), ranging from 1-4 per item. This gives a total maximum score of 64. The CHOP-INTEND score represents measures of disease severity. CHOP-INTEND scores are assessed in CL-101, CL-303, CL-304 cohort 1 and in CL-302.

Paediatric Neuromuscular Clinical Research Database (PNCR)

The PNCR natural history cohort, consisting of all patients (n=23) with age of onset ≤ 6 months, bi-allelic deletion of *SMN1* and 2 copies of *SMN2* for whom enrolment data (retrospective and prospective) were available was drawn[7] from The Paediatric Neuromuscular Clinical Research (PNCR) database, a natural history study of 337 patients with any form of SMA followed at 3 large, tertiary medical centres. Inclusion of the patients ranged from May 2005 to April 2009.
The PCNR database also contains data on patients with 3 SMN2 copies, which were not included in the PCNR natural history cohort but are used for comparison in study CL-304.

[7] Finkel et al, American Association of Neurology 2014.

Primary endpoints in the PNCR study were age at death and age at reaching the combined endpoint of either death or requiring at least 16 hours/day of non-invasive ventilation support for at least 14 days in the absence of an acute reversible illness or peri-operatively (as a surrogate for death). CHOP-INTEND scores and motor milestone achievement were obtained.

NeuroNext study

A population drawn from the NeuroNext natural history study (SMA type 1 patients with bi-allelic deletion of *SMN1* and 2 copies of *SMN2* were included in the comparator cohort (n=16)) was used as a supplementary control cohort for exploratory analyses in study CL-101. The NeuroNext natural history study was a longitudinal, multi-centre, prospective natural history study that enrolled 26 SMA infants <6 months of age at 14 centres over 21 months within the National Institute of Neurological Disorders and Stroke (NINDS) -sponsored NeuroNext Network[8] between December 14, 2012 and September 10, 2014.

Infant motor function as measured by CHOP-INTEND was assessed and CMAP measurements were obtained. Survival as defined in Study CL-101 was not captured in the NeuroNext study; survival within NeuroNext was defined as alive without tracheostomy.

Results

The following outcomes from the PCNR study are relevant for the comparison of the natural history cohort to the patients in the clinical studies:

Survival

Sixteen (16) patients in the PNCR cohort reached the combined event (death or the need of non-invasive ventilation support). The patient-level survival rate of this control cohort determined at 13.6 months of age from the Kaplan-Meier curve is approximately 25%, this rate will be used as comparator with ITT population for survival analysis. The median survival is 10.5 months.

When observed as a group, SMA type 1 patients with 3 copies of *SMN2* experienced a less severe clinical course when compared with the population of patients with 2 copies of *SMN2*; 25th percentile for survival was 22 months with median survival not observed due to insufficient events. Most patients experienced prolonged survival with only 17% mortality during observation and an age range of between 15 months and 52 years (median 7.4 years).

Motor Milestones

None of the patients in the PNCR control cohort reached the milestone of sitting without support for ≥10 seconds.

A proportion of patients with 3 copies of *SMN2* will fail to gain basic milestones such as independent sitting however the phenotypic variation of patients with 3 *SMN2* copies is large.

CHOP INTEND

No patient achieved a CHOP INTEND score >40 at or after the 6-month visit (with one transient exception).

The following outcomes from the NeuroNext study are relevant for the comparison of the natural history cohort to the patients in the clinical studies:

The data for the NeuroNext cohort reflects tracheostomy-free survival, a more lenient endpoint (a child could receive 24 hours per day of non-invasive support without triggering the combined endpoint). The

[8] Kolb et al, Annals of Clinical and Translational Neurology 2016 and Kolb et al, Annals of Neurology 2017.

percentage reaching the survival endpoint at 14 months of age is therefore slightly lower that in the PCNR cohort (62.5% of the patients versus 78.3% in the PNCR cohort).

In the NeuroNext cohort, no child achieved the milestone of sitting with or without support, hands and knees crawling, standing with assistance, walking with assistance, standing alone or walking alone.

In the NeuroNext cohort, no patient achieved a CHOP INTEND score >33 at or after the 6-month visit, and no patient had an increase in score from baseline. A mean decline of 10.7 points was observed between the 6 and 12 months of age visit.

Study CL-303

This is a Phase 3, open-label, single-arm, single-dose study of Zolgensma that enrolled 22 patients with SMA Type 1 who were symptomatic with no functional *SMN1* gene as well as 2 copies of *SMN2* and who are < 6 months (< 180 days) of age at the time of gene replacement therapy (Day 1). The study is completed but no final CSR was submitted for this study yet. Results of the requested preliminary analysis (cut-off 31 DEC 2019) are presented.

Methods

- **Study participants**

Main Inclusion criteria:

1. Patients with SMA Type 1 as determined by the following features: a. Diagnosis of SMA based on gene mutation analysis with bi-allelic *SMN1* mutations (deletion or point mutations) and 1 or 2 copies of *SMN2* (inclusive of the known *SMN2* gene modifier mutation (c.859G>C).
2. The first three patients enrolled must meet the criteria for the Intent-To-Treat population (see statistical analysis section).
3. Patients must be < 6 months (< 180 days) of age at the time of Zolgensma infusion.
4. Patients must have a swallowing evaluation test performed prior to administration of gene replacement therapy.

Main Exclusion criteria:

1. Previous, planned or expected scoliosis repair surgery/procedure during the study assessment period.
2. Pulse oximetry < 96% saturation at screening while the patient is awake or asleep without any supplemental oxygen or respiratory support, or for altitudes > 1000 m, oxygen saturation < 92% awake or asleep without any supplemental oxygen or respiratory support. Pulse oximetry saturation may decrease to < 96% after screening provided that the saturation does not decrease by ≥ 4 percentage points.
3. Tracheostomy or current use or requirement of non-invasive ventilatory support averaging ≥ 6 hours/day over the 7 days prior to the screening visit; or ≥ 6 hours/day on average during the screening period or requiring ventilatory support while awake over the 7 days prior to screening or at any point during the screening period prior to dosing.
4. Patients with signs of aspiration/inability to tolerate non-thickened liquids based on a formal swallowing test performed as part of screening. Patients with a gastrostomy tube who pass the swallowing test will be allowed to enrol in the study.

- **Treatments**

Patients will receive a one-time dose intravenous of Zolgensma at 1.1 X 10^{14} vg/kg, equivalent to the dose received by Cohort 2 in the Phase 1 study.

Patients were pre-treated with corticosteroids:

- Pre-treatment with oral prednisolone should always be given 24 hours prior to infusion at a dose of 1 mg/kg/day
- Following 30 days of prednisolone treatment, the 1 mg/kg/day dose can be tapered over 4 weeks for patients whose alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values are both below 2 × upper limit of normal (ULN) (e.g., 2 weeks at 0.5 mg/kg/day and then 2 weeks at 0.25 mg/kg/day).
- If both the AST and ALT values remain > 2 × ULN after 30 days of prednisolone treatment, the 1 mg/kg/day dose should be continued until the values return to normal range.

- **Objectives/ Endpoints**

Efficacy:

Co-Primary

- Proportion of patients that achieve functional independent sitting for at least 30 seconds at the 18 months of age study visit. It is defined by the Bayley Scales of Infant and Toddler Development (Version 3), confirmed by video recording, as a patient who sits up straight with head erect for at least 30 seconds.
- Survival at 14 months of age (see study CL-101).

Co-Secondary

- The proportion of patients maintaining the ability to thrive, defined as the ability to tolerate thin liquids (as demonstrated through a formal swallowing test) and to maintain weight (> third percentile based on World Health Organization [WHO] Child Growth Standards for age and gender) without need of gastrostomy or other mechanical or non-oral nutritional support at 18 months of age
- The proportion of patients who are independent of ventilatory support, defined as requiring no daily ventilator support/usage at 18 months of age, excluding acute reversible illness and perioperative ventilation, as defined above through assessment of actual usage data captured from the device (Phillips Trilogy)

Exploratory

- Proportion of patients that achieves motor milestones (see CL-101): the ability to hold head erect without support, roll from back to both sides, to sit with support independently (> 10 seconds; WHO), ability to crawl, ability to pull to stand, ability to stand with assistance, to stand alone, to walk with assistance, to walk alone
- Improvement of raw score from baseline to the maximum using Bayley Scales of Infant and Toddler Development (Version 3), Fine and Gross Motor Function Subtests
- Change from baseline to maximum CHOP-INTEND score.

- **Sample size**

Enrolling up to twenty (20) patients under the broader enrolment criteria is projected to enable enrolment of at least fifteen (15) patients that meet the Intent-to-Treat Population (ITT) criteria. The ITT population is identified as symptomatic patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2* without the *SMN2* gene modifier mutation (c.859G>C) who meet all other study enrolment criteria. In addition, the first three patients enrolled must meet the criteria for the Intent-to-Treat Population to enable a comparison of CHOP-INTEND scores at one-month following gene replacement therapy to enable a comparison of patient response between CL-303 patients and Cohort 2 patient results from the Phase 1 trial (CL-101).

- **Randomisation**

Not applicable

- **Blinding (masking)**

Not applicable

- **Statistical methods**

The study has been completed with key efficacy results provided as a part of 31 December 2019 update. Final CSR will be provided in September 2020. The primary and secondary efficacy endpoints will be compared to the null. The survival co-primary efficacy variable will be evaluated relative to literature-based historical controls.

Results

Participant flow

As of the cut-off date of 31 DEC 2019, 22 patients were enrolled in Study 303.
One patient was discontinued due to death (caused by respiratory distress not considered related to study drug) on Study Day 171 at the age of 7.8 months. One patient withdrew from the study and was alive but needed ventilation assistance ($\geqslant$ 16 hours of non-invasive BiPAP ventilator support for $\geqslant$ 14 consecutive days) at the EOT visit. One patient withdrew consent due to an AE just before the end of study visit at 18 months of age. Therefore 19 patients completed the study. All 22 patients met the ITT criteria, which are symptomatic patients with bi-allelic *SMN1* deletions and 2 copies of *SMN2* without the *SMN2* gene modifier mutation (c.859G>C) who receive an IV infusion of Zolgensma at < 180 days of age.

Baseline data

Key demographic and baseline characteristics for 22 patients enrolled in Study CL-303 are summarized in

Table 10. Twelve female patients (54.5%) and 10 male patients (45.5%) were enrolled in the study and received an infusion of Zolgensma. Nineteen of these patients were symptomatic and had homozygous deletion of *SMN1* and 2 copies of *SMN2*. One patient was asymptomatic and had homozygous deletion of *SMN1* and 2 copies of *SMN2* but was later re-classified as being symptomatic.

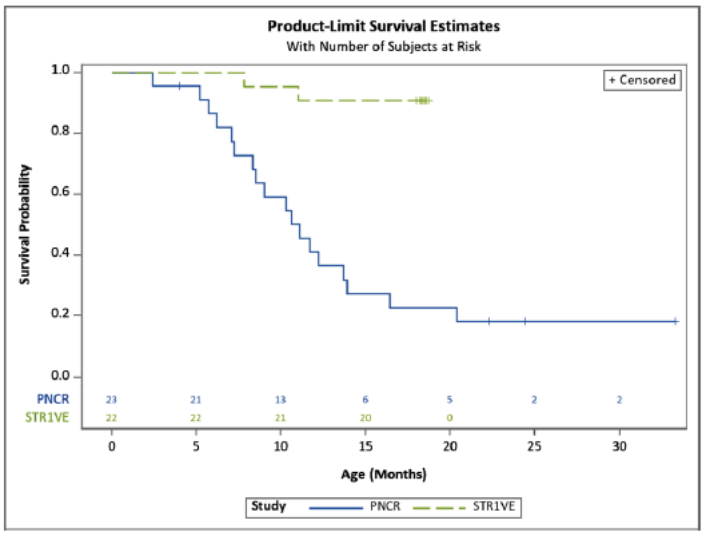
Table 4: Demographic and baseline characteristics of patients in study CL-303.

Category/Statistic	(N = 22)
Age at Day 0 (months)	
Mean (SD)	3.73 (1.61)
Median	3.50
Min, Max	0.5, 5.9
Sex, n (%)	
Male	10 (45.5)
Female	12 (54.5)
Race, n (%)	
White	11 (50.0)
Black or African American	3 (13.6)
Asian	2 (9.1)
Other	6 (27.3)
Ethnicity, n (%)	
Not Hispanic or Latino	18 (81.8)
Hispanic or Latino	4 (18.2)
Weight at baseline (kg)	
Mean (SD)	5.82 (1.05)
Median	5.80
Min, Max	3.9, 7.5
Gestational age at birth (weeks)	
n	22
Mean (SD)	39.0 (0.95)
Age at symptom onset (months)	
Mean (SD)	NAV
Swallowing thin liquid, n (%)	
Yes	22 (100.0)
No	0
Non-oral feeding support, n (%)	
Yes	0
No	22 (100.0)
Ventilatory support (invasive/non invasive), n (%)	
Yes	0
No	22 (100.0)
CHOP-INTEND score at baseline, n (%)	
Mean (SD)	32.0 (9.9)
Median	33,5
Min, Max	17, 52

Outcomes and estimation

Survival

The age at baseline ranged from 0.5 to 5.9 months. Twenty (20) of 22 patients (90.9%) met the co-primary endpoint of survival at 14 months of age. Thus, Zolgensma compares favorably to PNCR natural history where 25% were alive at 13.6 months. The co-primary endpoint survival was defined by either death or requiring permanent ventilatory support. The favorable 14 months of age survival data is substantiated by the co-secondary endpoint of ventilatory independence at 18 months of age: 18 of 22 patients (81.8%) were independent of ventilatory support (as assessed by Trilogy BiPAP data, see section on non-invasive and ventilator support below).Thus, Zolgensma compares favourably to PNCR natural history where 25% survived at 13.6 months.



Source: Study CL-303 Figure 14.2.4.1.1

Figure 4: Kaplan Meier plot for Event free Survival in study CL-303

Motor milestones

The video confirmed developmental milestones achieved in Study 303 are listed in Table 11.

Table 5: Developmental Motor Milestone Achievement in Study 303 (31 DEC 2019 cut off)

Video documented milestone	**Number of patients achieving milestone n/N (%)**	**Median age to the milestone achievement (Months)**
Head control*	17/20 (85)	6.8
Rolls from back to sides	13/22 (59)	11.5
Sits without support for 30 seconds (Bayley)	14/22 (64)	12.5
Walking alone	1/22 (64)	15.3

*2 patients were reported to have Head Control by clinician assessment at baseline.

The co-primary endpoint of sitting independently for at least 30 seconds at 18 months of age was met by 13/22 patients (659).

CHOP-INTEND scores

CHOP-INTEND scores at 18 months of age (end of study visit) were available for 16/22 patients. One patient deceased and two discontinued prior to 18 months of age visit, while three patients had no CHOP-INTEND examinations in line with the protocol due to prior three consecutive CHOP-INTEND scores ≥ 58. The mean total score (SD) was 51.2 (5.67) and the mean change from baseline (SD) was 19.3 (9.13).

All patients showed increase in CHOP-INTEND score from baseline during the study. The mean (SD) increases (improvement) from baseline to Month 1, Month 3, Month 6 and Month 12 after the administration of Zolgensma were 6.9 (5.35), 11.7 (6.40), 14.6 (7.04) and 16.4 (8.04) points, respectively.

Twenty-one patients (95.5%) achieved a CHOP-INTEND score of ≥40, 14 (63.6%) achieved a score of ≥50 and 5 patients (22.7%) achieved a score ≥60 at any point of time in the study.

The individual last recorded scores and changes from baseline are displayed in Table 12 and Figure 5.

Table 6: individual CHOP-INTEND scores in study CL-303

patient number	last recorded CHOP-INTEND score	Age recorded (months)	baseline	change from baseline
1	45	7.1	18	27
2	55	18.4	34	21
3	58	18.5	47	11
4	58	18.5	38	20
5	46	18.3	26	20
6	54	18.3	21	33
7	50	18.4	36	14
8	42	16.5	27	15
9	62	14.4	36	26
10	58	18.4	21	37
11	53	18.4	35	18
12	52	18.3	43	9
13	50	18.3	26	24
14	47	18.2	22	25
15	49	18.5	27	22
16	44	18.3	40	4
17	40	18.6	33	7
18	59	18.5	44	15
19	64	6	52	12
20	29	11	24	5
21	58	16.6	36	22
22	46	18.6	18	28

a: patients discontinued or were withdrawn from the study before the end of study visit.
Per protocol, when patients reached a score of ≥58, CHOP-INTEND assessment were not continued.

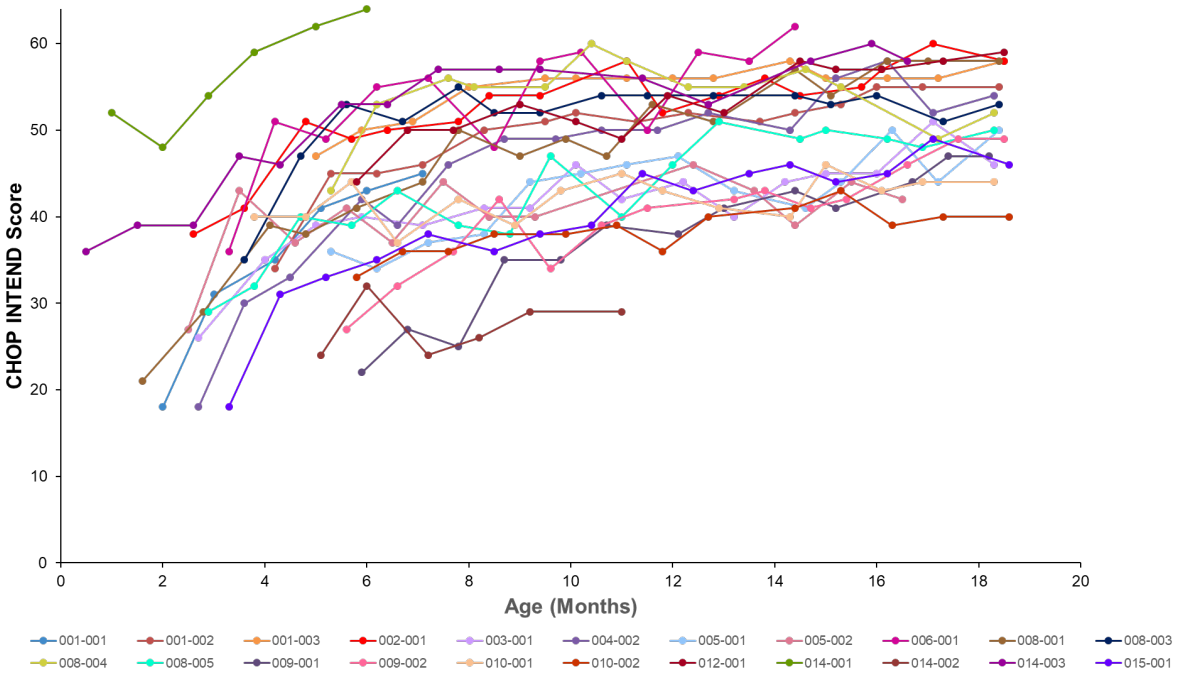


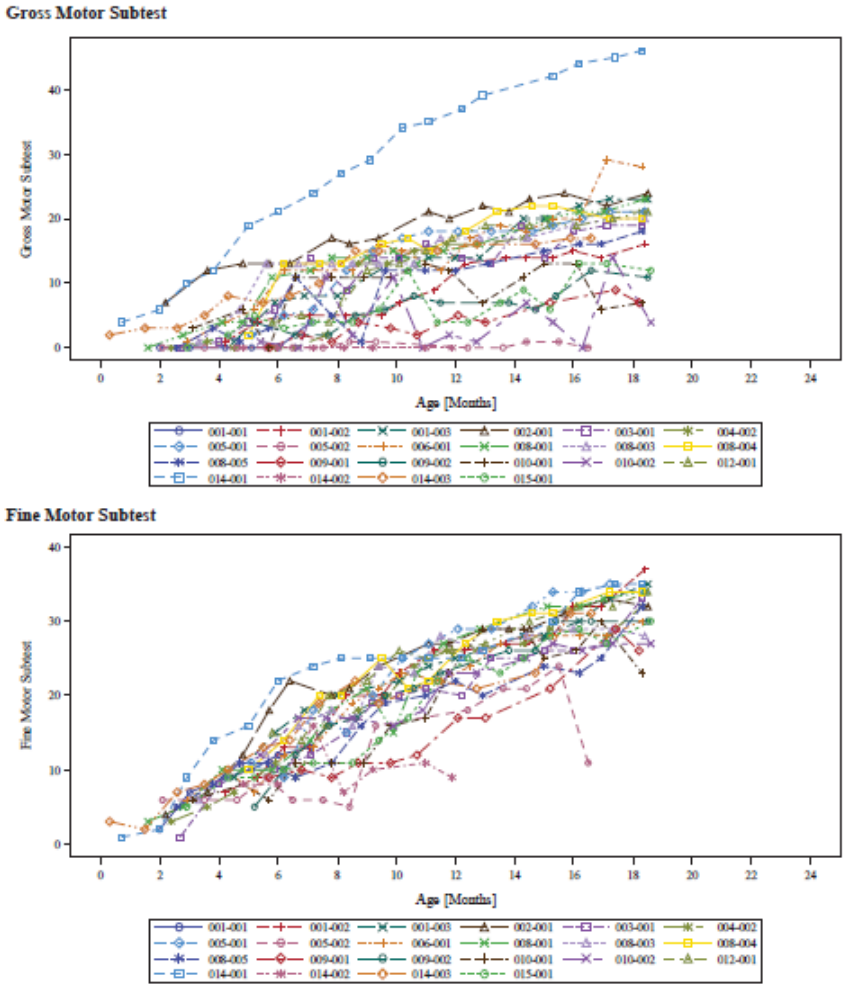
Figure 5: CHOP-INTEND Scores in study CL-303

Bayley Scales of Infant and Toddler Development

The gross and fine motor subtests of the Bayley Scales of Infant and Toddler Development Third Edition (an assessment tool of developmental function in children between the ages of 1 to 42 months) were administered at baseline and then monthly beginning with Visit 6 in Study 303.

The mean change from baseline to the highest post-baseline value was mean (SD) 23.9 (6.60) for the raw fine motor scale and 16.0 (9.19) for the raw gross motor scale. The raw scores are presented in (Figure 6) Scaled scores over time were also evaluated in Study CL-303. The scaled score reflects performance according to age as compared with other, normally developing children of the same age. The mean score is 10, with standard deviation of ± 3 points; thus, approximately 97% of children tested will fall within 2 standard deviations of the mean. In the gross motor subtest patients in CL303 had a mean (SD) baseline scaled score of 2.3 (2.55). The mean gross motor scaled score remained effectively unchanged through 18 Months (End of study), when the mean (SD) was 1.3 (1.41). In the fine motor subtest patients had mean (SD) scaled score of 6.4 (2.90) at baseline, and this also remained effectively unchanged through 18 Months (end of study) when the mean (SD) was 7.7 (3.16). Therefore, on average Study CL-303 patients displayed gross motor function that was significantly lower than in same-age peers, but tracking developmental gains. Fine motor function was largely similar (within 2 SD) to same-age peers.

The cognitive and expressive communication domains were also assessed within study CL-303 but no analysis of these domains is submitted.



Source: Study CL-303 Figure 14.2.4.2.1

Figure 6: Bayley Scales Gross and fine Motor Subtests (Raw Scores) in Study Zolgensma-CL-303 (ITT Population)

Obese

Non-invasive and Invasive Ventilatory Support and Normal Feeding Support

18 of 22 patients (81.8%) were independent of ventilatory support (as assessed by Trilogy BiPAP data) at 18 months of age (co-secondary endpoint, p< 0.0001). Four patients did not achieve independence of ventilatory support (as assessed by Trilogy BiPAP data) at 18 months of age. Two (2) patients had Trilogy data at or after 18 months of age and two patients withdrew from the study prior to 18 months of age. In total, 15 of 22 patients (68.1%) did not require any non-invasive ventilatory support at any point during the study.

Fifteen (15) of 22 patients (68.1%) received no non-oral feeding support at any time during the study. Seven patients (31.8%) received non-oral feeding support at some point during the study. Of these, four patients had intermittent or transient feeding support during the study and were not receiving non-oral feeding support at the end of the study. A total of 19 of 22 patients (86.3%) were feeding without mechanical support at the end of the study (or early termination). Two (2) patients had G-tube placement and were receiving feeding support at the end of the study or withdrawal from the study. One (1) patient discontinued prematurely from the study and feeding support was ongoing at the time of withdrawal.

The ability to thrive at 18 months of age was a co-secondary endpoint defined as the ability to tolerate thin liquids, does not receive nutrition through mechanical support, and maintains weight consistent with age. Nine of 22 patients (40.9%) met the ability to thrive criteria at 18 months of age (

Table 13).

Table 7: Proportion of Patients with the Ability to Thrive at 18 Months of Age (ITT Population)

	N = 22
Subitems comprising the ability to thrive at 18 months of age	
Ability to tolerate thin liquids	12 (54.5)
Does not receive nutrition through mechanical support	19 (86.4)
Maintains weight consistent with age	14 (63.6)
Maintain ability to thrive at 18 months of age	
n (%)	9 (40.9)
97.5% CI*	(18.6, 66.4)
p-value*	<0.0001

*P-value and 97.5% confidence interval are from a one-sided exact binomial test.
Source: Study CL-303 Table 14.2.2.1

Table 8: Summary of main efficacy results (Study CL-303).

Title: PHASE III GENE TRANSFER CLINICAL TRIAL FOR SPINAL MUSCULAR ATROPHY TYPE 1			
Study identifier	CL-303		
Design	Phase 3, open-label, single-arm, single-dose study of Zolgensma		
	Duration of study:		Until patients reach 18 months of age
Hypothesis	Superiority over best standard of care (natural history)		
Treatment	Treatment cohort		1.1E14 vg/kg, n=22
	Natural history control cohort		Best standard of care, n=23
Endpoints and definitions	Co-Primary endpoints	Survival	Survival at 14 months of age, defined as time from birth date to either (a) death or (b) permanent ventilation, defined as requirement of invasive ventilation or ≥16 hours of respiratory assistance per day (including non-invasive ventilatory support) continuously for ≥14 days in the absence of an acute reversible illness, excluding perioperative ventilation.
		Independent sitting	Proportion of patients that achieve functional independent sitting for at least 30 seconds at the 18 months of age study visit.
	Co-Secondary endpoints	Ability to thrive	The proportion of patients maintaining the ability to thrive, without need of gastrostomy or other mechanical or non-oral nutritional support at 18 months of age
		Ventilatory support	The proportion of patients who are independent of ventilatory support, defined as requiring no daily ventilator support/usage at 18 months of age
	Exploratory endpoint	Motor milestones	Demonstration of improvement of motor function and muscle strength as determined by achievement of significant development milestones during the study.

	Exploratory endpoint	CHOP-INTEND	Change from baseline to maximum CHOP-INTEND score.
	Exploratory endpoint	Bayley's scale	Improvement of raw score from baseline to the maximum using Bayley Scales of Infant and Toddler Development (Version 3), Fine and Gross Motor Function Subtests.
Data cutoff	31 DEC 2019		
NOTES	This table includes data from data cut off 31 dec 2019. No final CSR has been submitted yet.		

Results and Analysis

Description	**Primary Analysis**		
Analysis population and time point description	**Survival at 14 months of age** Intent to treat population		
Descriptive statistics	Treatment group	Study cohort	Natural history control cohort
	N	22	23
	Number of patients (%)	20 (90.9%)	5 (22%)

Analysis population and time point description	**Independent sitting at 14 months of age** Intent to treat population		
Descriptive statistics	Treatment group	Study cohort	Natural history control cohort
	N	22	23
	Number of patients (%)	13/22 (59%)	0 (0 %)

Description	**Secondary analysis**		
Analysis population and time point description	**Ventilatory support** ITT population		
Descriptive statistics	Treatment group	Study cohort	Natural history control cohort
	N	22	23
	Number of patients (%)	18/22 patients (81.8%)	0 (0 %)
Analysis population and time point description	**Ability to thrive** ITT population		
Descriptive statistics	Treatment group	Study cohort	Natural history control cohort
	N	22	23
	Number of patients (%)	9/22 patients (40.9%)	0 (0 %)

Description	**Exploratory endpoint: CHOP INTEND increase from baseline**	
		Mean (SD)
	Month 3 (n=22)	11.7 (± 6.40)
	Month 6 (n=20	14.6 (± 7.04)
	Month 12 (n=16)	16.4 (±8.04)

Description	Exploratory endpoint: Motor milestone achievement during the study	
		n/N
	Head control	17/20
	Rolling back to sides	13/22
	Sits alone >30 sec	14/22
	Walks alone	1/22

Ancillary analyses

Results by time interval of time of diagnosis to time of treatment

The applicant has provided an additional analysis of the possible relationship between maximum CHOP INTEND score after treatment and the time interval between SMA diagnosis and treatment. No significant correlation was found with regression analysis in study CL-303. However, significant methodological issues were identified. The applicant has committed to design a model predicting the expected treatment outcome post-marketing.

2.5.3. Supportive studies

Study CL-101 is a Phase 1, open-label, single-infusion, ascending-dose, single-centre study to evaluate the safety and efficacy of Zolgensma in up to 15 patients with Type 1 SMA. Primary endpoint was survival (time from birth to either death or permanent ventilation), analysed once all patients reached13.6 months of age. The most relevant secondary endpoints were motor milestone achievement and the change from baseline CHOP-INTEND score.

LT-001, the long-term follow-up study of study CL-101 is submitted to support the maintenance of efficacy.

Study CL-302 is an ongoing, Phase 3, open-label, single-arm, single-dose, trial of Zolgensma in patients with a biallelic mutations of the *SMN1* gene and with one or two copies of *SMN2*. Up to 30 patients < 6 months (< 180 days) of age at the time of gene replacement therapy (Day 1) were enrolled starting from Q2 2018. The study has been designed as the European counterpart of study CL-303. Nine European investigative sites in Belgium, France, Italy and the United Kingdom have currently enrolled patients for this study.

Study CL-304 is an ongoing, global, Phase 3, open-label, single-arm, single-dose, multicenter study of IV Zolgensma for the treatment of ≥ 44 pre-symptomatic newborn patients expected to develop Type 1, 2, or 3 SMA with 2 (cohort 1), 3 (cohort 2), or 4 copies of *SMN2* who are ≤ 6 weeks of age at the time of gene replacement therapy. Patients with *SMN1* point mutations or the *SMN2* gene modifier mutation (c.859G>C) are not excluded.

In addition, the applicant provided information on extended access programs and managed access programs existing in the United States and France, under which at least 51 patients up to the age of 29 months and up to 13.1 kg were treated with intravenously administered Zolgensma.

2.5.3.1. Study CL-101

Study Zolgensma-CL-101 was a Phase 1, open-label, single-infusion, ascending-dose, single-centre study to evaluate the safety and efficacy of Zolgensma in up to 15 patients with Type 1 SMA.

Methods

Main inclusion Criteria:

- Bi-allelic *SMN1* gene mutations (deletion or point mutation) with 2 copies of *SMN2* and excluding the c.859G>C modification in exon 7
- Patients 6 months and younger with disease onset up to 6 months of age.
- Hypotonia by clinical evaluation with delay in motor skills, poor head control, round shoulder posture, and hypermobility of joints.

Main exclusion criteria:

1. Patients with antibody to adeno-associated virus serotype 9 (anti-AAV9) titres >1:50 as determined by enzyme-linked immunosorbent assay binding immunoassay.
2. Patient with signs of aspiration based on a swallowing test and unwilling to use an alternative method to oral feeding.

The study included 2 sequential dosing cohorts:

- Cohort 1 (low dose): 6.7E13 vg/kg (3 patients)
- Cohort 2: 2.0E14 vg/kg (12 patients)

The primary efficacy endpoint was the time from birth to either

1. requirement of ≥16-hour respiratory assistance per day (includes bi-level positive airway pressure [BiPAP]) continuously for ≥2 weeks in the absence of an acute reversible illness, excluding perioperative ventilation or
2. death.

The key secondary endpoints that were evaluated included:

- The change from baseline in Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) score. The CHOP-INTEND is a physical therapy assessment designed and validated specifically for use with patients affected by SMA Type 1[9]. If a patient achieved 2 consecutive CHOP-INTEND scores ≥62, the PI, the physical therapist, and the Sponsor reviewed the patient status and determined whether or not continued CHOP-INTEND assessments were necessary, otherwise CHOP-INTEND assessments continued monthly during Year 1 and quarterly during Year 2. Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders examinations were video recorded.
- Motor milestone achievement was determined by an external reviewer.

Exploratory efficacy objectives presented to support the application:

- Bayley Scales of Infant and Toddler Development Third Edition (Bayley Scales), a standardized, norm-referenced infant assessment. The gross and fine motor portions as well as speech and cognition portions of this test were administered in patients who achieved a score ≥60 of 64 on the CHOP-INTEND. The Bayley Scales are a standardized, norm-referenced infant assessment of developmental functioning across 5 domains: cognitive, language, motor, social-emotional, and adaptive behaviour. The Bayley Scales are administered by a physical therapist. A scaled score of ≥8 on the Bayley Scales would be considered the low end of normal. Gross and fine motor

[10] Mercuri et al, Diagnosis and management of spinal muscular atrophy: Part 1: Recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. Neuromuscular disorders 2018.

portions were to be completed monthly through the time point that the patient reached 15 months of age or 12 months post-dose, whichever was later. The language and cognition portions were to be administered every 3 months.

- Achievement of the developmental milestone of functional independent sitting for at least 30 seconds and developmental milestones of sitting unassisted for 15 seconds, 10 seconds, and 5 seconds, respectively, for patients that did not achieve functional independent sitting for at least 30 seconds.
- The proportion of patients who did not require non-oral nutrition prior to therapy who maintained the ability to thrive, defined by the ability to tolerate thin liquids (as demonstrated through a formal swallowing test), did not receive nutrition through mechanical support (ie, feeding tube), and maintained weight (>3rd percentile for age and gender)
- The proportion of patients who were independent of ventilatory support, defined as requiring no daily ventilator support/usage in the absence of acute reversible illness and excluding perioperative ventilation.

Statistical methods

The CSR includes data from efficacy analyses conducted at the following time points:

- The primary efficacy data cut-off (20Jan2017), the date at which all patients had completed a study visit after reaching 13.6 months of age
- When the last enrolled patient had a study visit after reaching 20 months of age (07Aug2017)
- When all patients completed 24 months of post-dose follow-up (14Dec2017)

The change from baseline in CHOP-INTEND score was analysed by using mixed model repeated measures (MMRM). The model for the FAS included the change from baseline as the dependent variable, and fixed effects of Cohort visit, and baseline as covariate, and interactions of cohort-visit, baseline-visit. An unstructured (general) covariance structure was assumed initially to model the within-patient errors; however, if unstructured covariance resulted in non-convergence, the variance component was used. The least squares means, differences between least squares (LS) means, a 95% 2-sided confidence limits for each difference and the p-values from model effects were reported for each scheduled visit. A post hoc analysis of Cohort 2 CHOP-INTEND scores using intrinsic factors was also conducted and reported separately.

The number (%) of patients who exhibited evidence of milestone achievement based on external expert confirmation by the time of efficacy data cut-off was summarized by Cohort using the FAS. The observed proportion attaining these milestones was compared to zero (the expected rate of attainment amongst untreated patients with SMA Type 1) using a 1-sided exact binomial test.

Results

Of the 16 patients who were screened for the study, 1 failed screening (due to exclusionary baseline AAV9 antibody titre) and 15 were enrolled and treated (3 in Cohort 1, 12 in Cohort 2).

None of the 15 treated patients discontinued from the study.

Baseline data

Table 9: Summary of Demographic and Baseline Characteristics (Study Zolgensma-CL-101) (Safety Analysis Set)

Statistic	Low Dose (Cohort 1) (N = 3)	Proposed Therapeutic Dose (Cohort 2) (N = 12)	All Patients (N = 15)
Age at Day 0[a] (months)			
Mean	6.3	3.4	4.0
Median	5.9	3.1	4.1
Minimum, Maximum	5.9, 7.2	0.9, 7.9	0.9, 7.9
Sex, n (%)			
Male	1 (33.3)	5 (41.7)	6 (40.0)
Female	2 (66.7)	7 (58.3)	9 (60.0)
Race, n (%)			
White	3 (100.0)	11 (91.7)	14 (93.3)
Other	0	1 (8.3)	1 (6.7)
Ethnicity, n (%)			
Not Hispanic or Latino	3 (100.0)	10 (83.3)	13 (86.7)
Hispanic or Latino	0	2 (16.7)	2 (13.3)
Gestational age at birth (weeks)			
N	2	10	12
Mean	39.0	38.5	38.6
Age at symptom onset (months)			
Mean	1.7	1.4	1.5
Weight at baseline (kg)			
Mean	6.6	5.7	5.9
Patients reported swallowing thin liquid, n (%)			
Yes	0	4 (33.3)	4 (26.7)
No	3 (100.0)	8 (66.7)	11 (73.3)
Patients reported non-oral feeding support, n (%)			
Yes	3 (100.0)	5 (41.7)	8 (53.3)
No	0	7 (58.3)	7 (46.7)
Patients reported ventilator support (invasive/noninvasive), n (%)			
Yes	3 (100)	2 (16.7) b	5 (33.3) b
No	0	10 (83.3)	10 (66.7)
Baseline CHOP-INTEND score, mean (range) (Full Analysis Set)	16.3 (6-27)	28.2 (12-50)	NAV (650)

Not all baseline characteristics were collected at the start of study, some patients may have incomplete data.
a Day of Zolgensma administration.
b Does not include one additional patient in Cohort 2 who was receiving BiPAP at baseline but for whom data was mis-entered at the clinical site.
Source: Table 14.1.2-24

Outcomes and estimation

Primary endpoint: Survival without permanent ventilation

Primary data cut-off: 13.6 months of age

All 15 patients (100%) survived without permanent ventilation through 13.6 months of age. Results in the ITT analysis set are provided in Table 16

Compared with the natural history estimate of 25%, all Zolgensma-treated patients had statistically significant improved survival without permanent ventilation.

Table 10: Proportion of Patients Who Survived Without Permanent Ventilation Up to 13.6 months of Age

Survival at 13.6 months		Comparison to survival rate of 25% at 13.6 months	
	n/N (%)	**2-sided 95.0% CI[a]**	**p-value[b]**
Cohort 1	3/3 (100)	29.24 ,100.00	0.016
Cohort 2	12/12 (100)	73.54, 100.00	<0.001
All	15/15 (100)	78.20, 100.00	<0.001

a Confidence interval from the superiority 1-sided exact binomial test.
b Compared to the natural history estimate of 25% (1) using a 1-sample exact binomial test.

Source: Table 14.2.1.2.1-14.

At 24 months of follow-up post-dose, the end-of-study assessment, all patients were alive and all but one patient was free of permanent ventilation. Compared with the historical survival rate, the survival rates for patients treated with Zolgensma were statistically significantly higher for both cohorts and for all patients combined.

Secondary endpoint: change from baseline in CHOP-INTEND score

illustrates the change in CHOP-INTEND score over time by patient and by cohort at the final study date cutoff (24 months post-dose). All patients (100%) achieved improvement of at least 4 points from baseline function.

At Months 1 and 3, patients in Cohort 2 had mean increases from baseline of 9.8 and 15.4, respectively (n=12, both p<0.001), while patients in Cohort 1 had a mean increase of 0.3 at Month 1 and a decrease of -0.3 at Month 3 (both n=3, both p≥0.817).

The mean increase from baseline was 25.4 in cohort 2. In the analysis of the FAS, the mean change from baseline in Cohort 2 was statistically significant from baseline from Month 1 through Month 19 (all p<0.001).

Scores for patients in Cohort 2 increased rapidly after Zolgensma administration, were greater than those observed in Cohort 1, and were sustained over time.

When comparing the 2 cohorts, the overall LS mean difference in the change from baseline was 23.22 (95% CI, 20.36, 26.07, p<0.001) favouring Cohort 2. Improvement from baseline in Cohort 2 patients was significantly greater compared with Cohort 1 from Month 1 through Month 19 (all p≤0.026).

Eleven of 12 Cohort 2 patients achieved CHOP-INTEND scores ≥40, 10/11 patients achieved scores ≥50, and 3/11 patients achieved scores ≥60 during the study (all 1-sided p<0.001 vs zero in the natural history cohort).

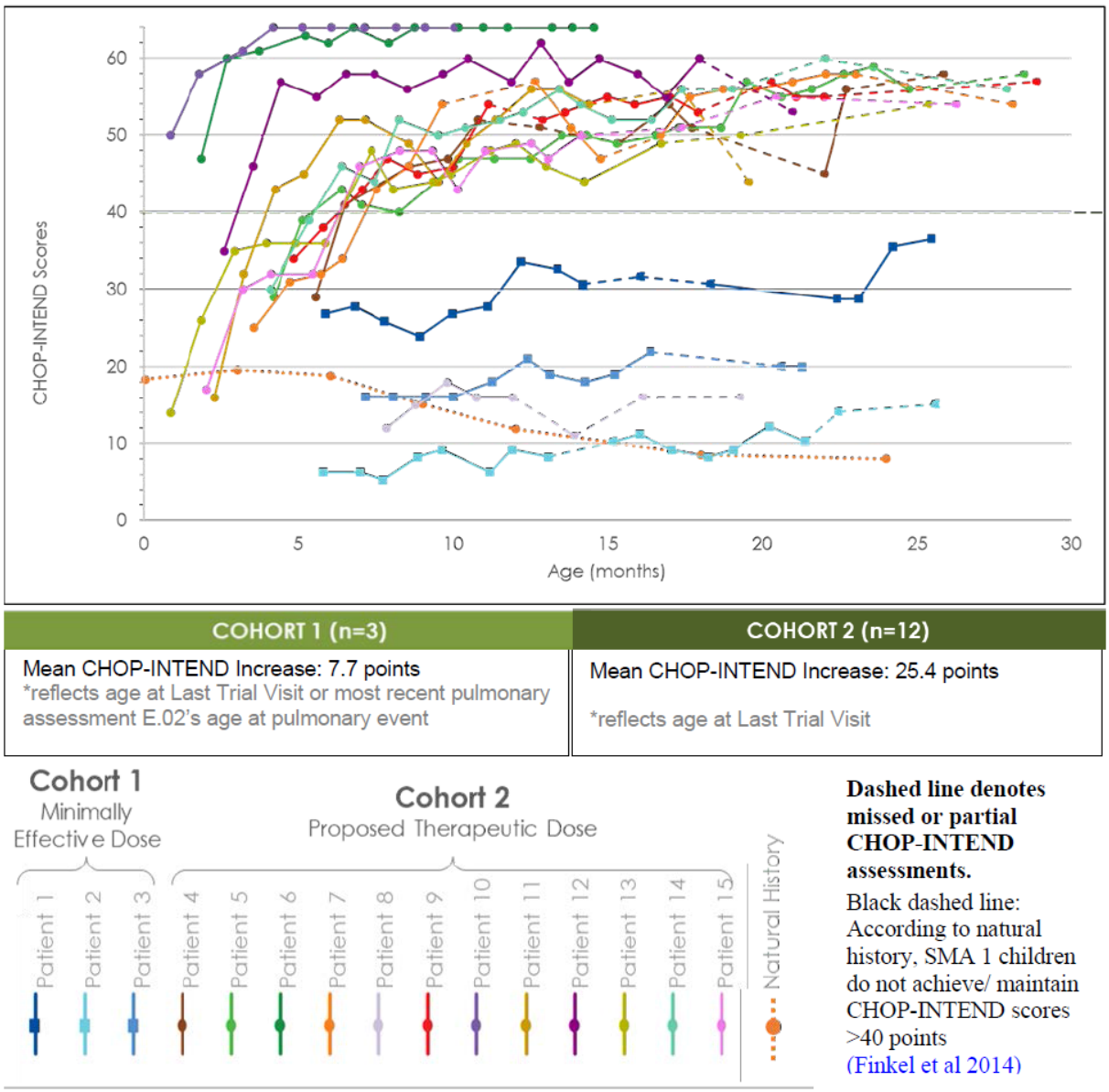


Figure 7: CHOP-INTEND Change from Baseline by Patient and Cohort at 24 Months of Follow-up Post-dose (Study Zolgensma-CL-101) (Full Analysis Set)

Secondary endpoint: Development of Significant motor function milestones

Primary data cut-off: 13.6 months of age

Table 12 represents the achieved milestones in cohort 2 at the different efficacy analysis time points. No patients in Cohort 1 achieved a significant motor milestone.

Final study data at 24 months post dose follow up

Table 11: Milestone achievement in Cohort 2 patients (n=12) as recorded in the three main study visits.

	13.6 months of age	20 months of age	24 months FU
Rolling (back to side from both sides)	9	9	9
Hold head erect ≥ 3 seconds, unsupported	11	11	11
Sits with support, non-independent sitting[a]	11		11
Sits without support ≥5 seconds[a,b]	9	11	11
Sits without support ≥ 10 seconds[a,c]	7	10	10
Sits without support ≥ 15 seconds[a,d]	6		9
Sits without support ≥ 30 seconds[a,d]	5	9	9
Stands with assistance	2		2
Stands alone	2	2	2
Walks with assistance	2		2
Walks alone	2	2	2

a Patients are included in multiple categories for the "sits without support" milestone, including patients who are observed sitting alone for ≥ 5, ≥ 10, ≥ 15, or ≥ 30 seconds. Patients sitting ≥ 30 seconds are included in the totals for ≥ 15 seconds, ≥ 10 seconds, and ≥ 5 seconds (WHO definition).
b This assessment is based on the Bayley Scales of Infant and Toddler Development® Third Edition (Bayley Scales): Gross and Fine Motor Skills subtests, item No. 22, "Sits Without Support Series: 5 seconds." Note: The source tables in the appendix for the milestones provided by central review include a milestone identified as "Sits without support < 10 seconds." The external reviewer has confirmed that this milestone was defined as "Sits without support ≥ 5 seconds" as is labeled here.
c This assessment is based on the World Health Organization (WHO)-Multicentre Growth Reference Study (MGRS) Guidelines.
d This assessment is based on the Bayley Scales: Gross and Fine Motor Skills subtests, item No. 26, "Sits Without Support Series: 30 seconds." Note: The Bayley Scales do not have a category for 15 seconds.

Source: Study CL-101 CSR Table 14.2.3.3.1-14 and Listing 16.2.15-14 in Module 5.3.5.1.

Although presented by the Applicant as an exploratory endpoint, the proportion of patients achieving and maintaining functional independent sitting (≥30 Seconds) 24 Months after Zolgensma infusion, is presented here for clarity since this endpoint was also determined by an external reviewer based on video recordings.

Table 12: proportion of patients Achieving and Maintaining Functional Independent Sitting (≥30 Seconds) 24 Months after Zolgensma Infusion.

Independent sitting at 24 months post-dose		Comparison to 0% in the natural history	
	n/N (%)	**2-sided 95.0% CI (%)**	**p-value[a]**
Cohort 1	0/3	0.00, 70.76	1.000
Cohort 2	9/12	42.81, 94.51	<0.001
All	9/15	32.29, 83.66	<0.001

a Observed percent compared to zero using a 1-sided exact binomial test. To make computation of the p-value possible, the value of 0.1% was used in place of a literal zero.

Source: Table 14.2.1.1.1-24 and Table 14.2.1.1.2-24

Bayley Scales

Three patients from Cohort 2 scored ≥60 on the CHOP-INTEND, and Bayley Scales assessments were initiated per protocol (initial assessments ranging from Day -1 to Day 236). Scores increased in all 3

patients from the first Bayley Scales assessment to the primary efficacy data cutoff, as illustrated in Figure 8.

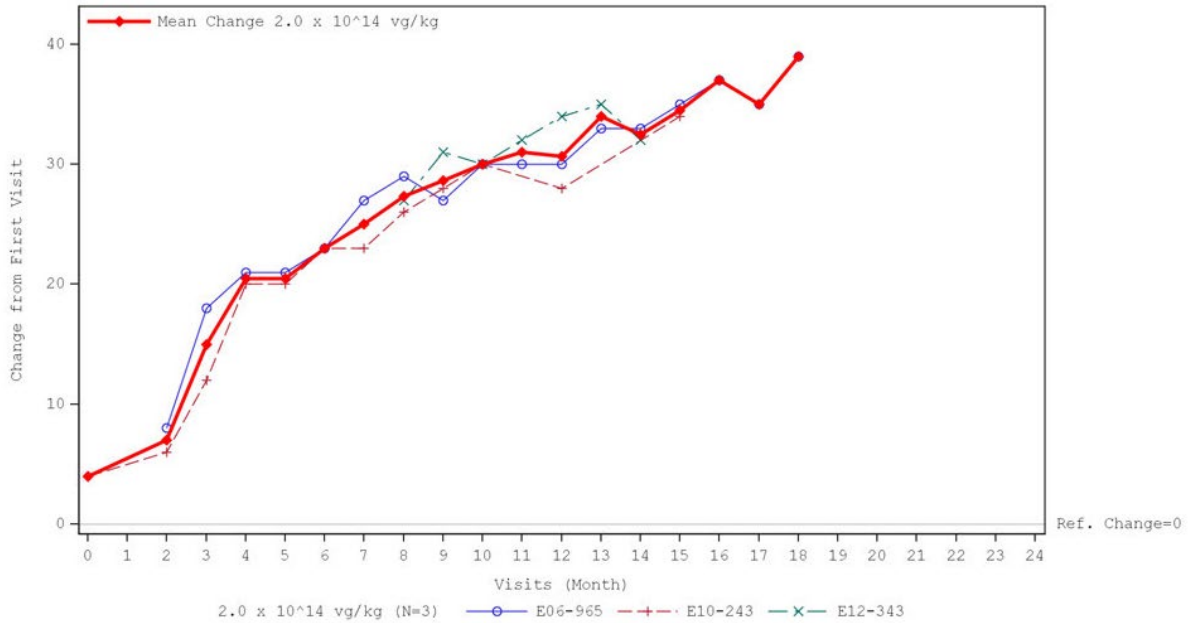


Figure 8: Spaghetti Plot of Bayley Scale Change for Infant and Toddler Development Over Time for All Patients by Cohort – Fine Motor Scale (Full Analysis Set).

Maintaining Ability to Thrive

The ability to thrive ITT analysis set included 7 patients from cohort 2 with bi-allelic deletion of *SMN1* and a baseline CHOP-INTEND score ≥20 at baseline and who did not require non-oral nutrition prior to administration of Zolgensma. Six of 7 patients (85.7%) maintained the ability to thrive at the 14 months of age study visit defined by the ability to tolerate thin liquids (as demonstrated through a formal swallowing test), did not receive nutrition through mechanical support (ie, feeding tube), and maintained weight (>3rd percentile for age and gender). At the 24 months post-dose study visit, 5 out of 7 patients maintained the ability to thrive (Table 19).

Table 13: Proportion of Patients Who Maintained the Ability to Thrive 24 Months After Zolgensma Infusion (Ability to Thrive ITT Analysis Set).

Ability to thrive at 24 months post-dose		Comparison to 0% in the natural history	
	n/N	**2-sided 95.0% CI (%)**	**p-value[a]**
Cohort 1	--	--	--
Cohort 2	5/7	29.04, 96.33	<0.001
All	5/7	29.04, 96.33	<0.001

a Observed percent compared to zero using a 1-sided exact binomial test. To make computation of the p-value possible, the value of 0.1% was used in place of a literal zero.

Source: Table 14.2.2.1-24

Pulmonary and Nutritional Achievements

Ten patients in cohort 2 were free of non-invasive ventilatory support (NIV) at baseline. During the course of the study, all patients in cohort 1 and 6 patients in cohort 2 were using NIV. The average hours per day was fairly stable, expect for some transient increases during illnesses and respiratory tract infections.

Nearly all patients experienced respiratory illnesses that, in children with Type 1 SMA, typically result in tracheostomy or death. All but one patient in Study CL-101 survived respiratory hospitalisations without tracheostomy or the need for permanent ventilation

In Cohort 2, 7 patients did not receive enteral feeding prior to gene replacement therapy. One (1) of these 7 patients had short period of nutritional support post-gene-replacement therapy to assist wound healing surgery. Four of the 5 patients in Cohort 2 who received enteral feeding prior to gene replacement therapy could feed orally at end-of-study; thus, a total of 11 of the 12 patients in Cohort 2 were able to feed orally.

Ancillary analyses

Subgroup assessments on change from baseline for CHOP-INTEND score were done for age, weight, baseline CHOP-INTEND score, and gender using Cohort 2 (which received the proposed therapeutic dose) from Study CL-101 to assess whether factors could be identified that predict which patients will benefit most from treatment. These analyses were not predefined and therefore not included in the SAP. No rationale is provided for this subgroup definition and this subgroup definition is not in line with the efficacy subgroup definition provided by the applicant somewhere else.

The following subgroups were formed:

Gender	Female (n=7)	Male (n=5)
Age at time of dosing	< 3.1 months (n=6)	≥ 3.1 months (n=6)
Weight at dosing	< 5.45 kg (n=6)	≥ 5.45 kg (n=6)
baseline CHOP-INTEND scores	< 29 (n=5)	≥ 29 (n=7)

Results by Age/weight at Dosing

Since age and weight at dosing were correlated, the results from these two post hoc analyses are combined. Improvement from baseline in CHOP-INTEND scores was somewhat greater, particularly across earlier time points, for the younger patient subgroup, i.e. < 3.1 months of age. At 3 months post-dose, younger patients improved 19.3 ± 5.6 points (mean, standard deviation, LOCF), whereas older patients improved 11.5 ± 4.5 points. By end-of-study (24 months of follow-up post-dose), improvements in CHOP-INTEND scores were more similar between the 2 subgroups, 25.1 ± 10.3 points for the younger subgroup and 24.0 ± 10.1 points for the older subgroup (

Table 20). The CHOP-INTEND scores were improved from baseline (nominal $P < 0.05$ by post-hoc statistical testing) for both subgroups at all time-points.

Of the 9 patients in Cohort 2 who achieved functional independent sitting for ≥ 30 seconds within 24 months of follow-up post-dose, 6 were ≤ 3 months of age and 3 were between 3 and 6 months of age at the time of Zolgensma administration.

Table 14: CHOP-INTEND Scores and Achievement of Independent Sitting Within 24 Months of Follow-up for Patients in Cohort 2 stratified by age at dosing.

			Total CHOP-INTEND Score			
Age at Dosing (months)	**Patient number**	**Highest Achievement of Sitting Independently (Seconds)**	**Baseline (Day -1)**	**1 month After GT**	**3 months After GT**	**Final CHOP-INTEND Score (Study Day)[a]**
Patients ≤ 3 months of age at the Time of Zolgensma Administration						
0.9	1	≥ 30	50	58	64	64 (Day 275)
0.9	2	≥ 30	14[b]	26	36	54 (Day 734)
1.9	3	≥ 30	47	60	63	64 (Day 381)
2.1	4	≥ 30	17	30	32	54 (Day 727)
2.3	5	≥ 30	16	32	45	56 (Day 762)
2.6	6	≥ 30	35	46	55	55 (Day 640)
Patients > 3 months of Age at the Time of Zolgensma Administration						
3.6	7	≥ 15	25	31	34	54 (Day 736)
4.1	8	≥ 30	30	39	44	56 (Day 713)
4.2	9	≥ 30	29	39	41	58 (Day 729)
4.9	10	≥ 30	34	38	47	57 (Day 722)
5.6	11	≥ 5[c]	29	41	46	55 (Day 800)
7.9	12	NA	12	15	16	16 (Day 343)

NA = not applicable, ie, patient did not sit independently.
a CHOP-INTEND- assessments were discontinued once patients achieved higher functioning status; therefore, the Study Day of the final CHOP-INTEND score varied for each patient.
b Baseline assessment for Patient E13-842 was on Day -2.
c Patient 11 had early corrective surgery for congenital scoliosis which impacted the ability to sit independently.

Results by Baseline CHOP-INTEND Score

Since the CHOP-INTEND scale has a maximum score of 64, improvement from baseline in CHOP-INTEND scores was numerically greater in patients with lower CHOP-INTEND scores at baseline but showed substantial overlap with scores in patients with higher baseline CHOP-INTEND scores. At 3 months post-dose, the patients with lower baseline CHOP-INTEND scores improved 15.8 ± 10.0 points (mean, standard deviation, LOCF) while patients with higher CHOP-INTEND scores at baseline improved 15.1 ± 2.7 points. By end-of-study (24 months of follow-up post-dose), improvement in CHOP-INTEND scores in patients with lower CHOP-INTEND scores at baseline was 27.0 ± 13.6 points and was 22.9 ± 6.6 points in patients with higher CHOP-INTEND scores at baseline.

Results by Gender

Mean CHOP-INTEND scores for the 2 gender subgroups were similar at baseline (males 28.4, females 28.0). Improvement in baseline CHOP-INTEND scores was numerically greater in males than in females but showed substantial overlap between the two groups. By the end of the study (24 months of follow-up post-dose), male patients improved 22.3 ± 10.5 points and female patients improved 27.8 ± 8.7 points. The CHOP-INTEND scores were improved from baseline (nominal $P < 0.05$ by post-hoc statistical testing) for both subgroups at all time points.

Results by time interval of time of birth to time of treatment

The applicant has provided an additional analysis of the possible relationship between maximum CHOP INTEND score after treatment and the time between birth and treatment. The results of this analysis suggest that time between birth and Zolgensma treatment has a significantly linear effect on the maximum CHOP-INTEND total score achieved (p=0.0142).

2.5.3.2. Study LT-001

Study LT-001 is an ongoing, observational, long-term, safety follow-up study of patients who completed Study 101. Of the 15 patients from study CL-101, 13 patients were enrolled in study LT-001 (3 from cohort 1 and 10 from cohort 2). The study consists of an initial 5-year phase, during which patients are being seen annually for evaluation of long-term safety, followed by a 10-year observational phase with annual telephone contacts.

The primary objective is to collect long-term safety data of patients with Type 1 SMA who were treated with Zolgensma in the CL-101 gene replacement therapy clinical trial.

An efficacy objective was included to determine whether the highest achieved milestone in Study CL-101 was maintained.

Motor milestones will be recorded at study initiation and at the annual study visits during the initial 5-year follow up according to the following checklist and will be supported by video documentation:

Current Status: Achieved	**Developmental Milestone: Based on Bayley Scales of Infant and Toddler Development and WHO-MGRS**
YES/NO	Child holds head erect for at least 3 seconds without support
YES/NO	Sitting with support
YES/NO	Sitting without support
YES/NO	Ability to crawl
YES/NO	Pulls to stand
YES/NO	Stand with assistance
YES/NO	Stand alone
YES/NO	Walk with assistance
YES/NO	Walk alone

Results

As of 31 DEC 2019, 13 patients had enrolled in Study LT-001 and had a baseline visit. Eleven patients had completed a 1-year post-baseline visit and 7 patients a 2-year post-baseline visit

Nusinersen is documented as having been used by 7 of the 13 enrolled patients (53.8%) at the 1-year visit. Thus, 3 of the 3 patients (100%) enrolled in Cohort 1 treated with the low dose of Zolgensma have been started on nusinersen and this is reported as ongoing (Study LT-101). Four of 10 patients (40.0%) who received the higher dose in Cohort 2 and who enrolled in Study LT-001 have been started on nusinersen. Nusinersen use is reported as ongoing for all patients. It is to be noted that nusinersen treatment was started on parental request to see if the children could achieve additional benefit from combination therapy. The limited dataset does not allow conclusions on additional benefit of nusinersen in Study LT-001 or lack thereof.

All 13 (100%) of the patients who enrolled in Study LT-001 were surviving (alive without permanent ventilation) as of 31 DEC 2019. All of the 10 higher-dose Cohort 2 patients (100%) enrolled in Study LT-001 have remained free of permanent ventilation. Two of the 3 patients (66.7%) in the lower dose Cohort 1 remain free of permanent ventilation. Five of the 10 enrolled Cohort 2 patients (50.0%) require no respiratory support and one Cohort 2 patient (33.3%) requires respiratory support only when ill. Thus, 6 of 10 enrolled higher-dose Cohort 2 patients (60.0%) require no regular, daily respiratory support.

New video-confirmed motor milestones were achieved by 2 patients: stands with assistance. Both of these patients have had a one-year follow-up visit and neither of these patients are reported to have used nusinersen.

Study CL-302

Study CL-302 is a Phase 3, open-label, single-arm, single-dose, trial of Zolgensma (gene replacement therapy) in patients with spinal muscular atrophy (SMA) Type 1 who meet enrolment criteria and are genetically defined by a biallelic mutation in *SMN1* gene and with one or two copies of *SMN2*. Up to 30 patients < 6 months (< 180 days) of age **at the time** of gene replacement therapy (Day 1) will be enrolled starting from Q2 2018. The study has been designed as the European counterpart of study CL-303. Nine European investigative sites in Belgium, France, Italy and the United Kingdom have currently enrolled patients in this study.

Objectives:

Primary

• Determine efficacy by demonstrating achievement of developmental milestone of sitting without support for 10 seconds up to 18 months of age as defined by WHO Motor Developmental Milestones.

Secondary

• Determine efficacy based on survival at 14 months of age. Survival is defined by the avoidance of combined endpoint of either (a) death or (b) permanent ventilation which is defined by tracheostomy or by the requirement of ≥ 16 hours of respiratory assistance per day (via non-invasive ventilatory support) for ≥ 14 consecutive days in the absence of an acute reversible illness, excluding perioperative ventilation. Permanent ventilation, so defined, is considered a surrogate for death.

Exploratory

• Achievement of motor milestones, CHOP-INTEND scores and Bayley's scale scores are amongst the exploratory endpoints of this study.

Results

Survival

At data cut, 32 of the 33 enrolled patients (93.6%) had had survived without permanent ventilation of which only 18 patients (56.3%) were ≥ 14 months of age and 4 (12.5%) were ≥ 18 months of age.

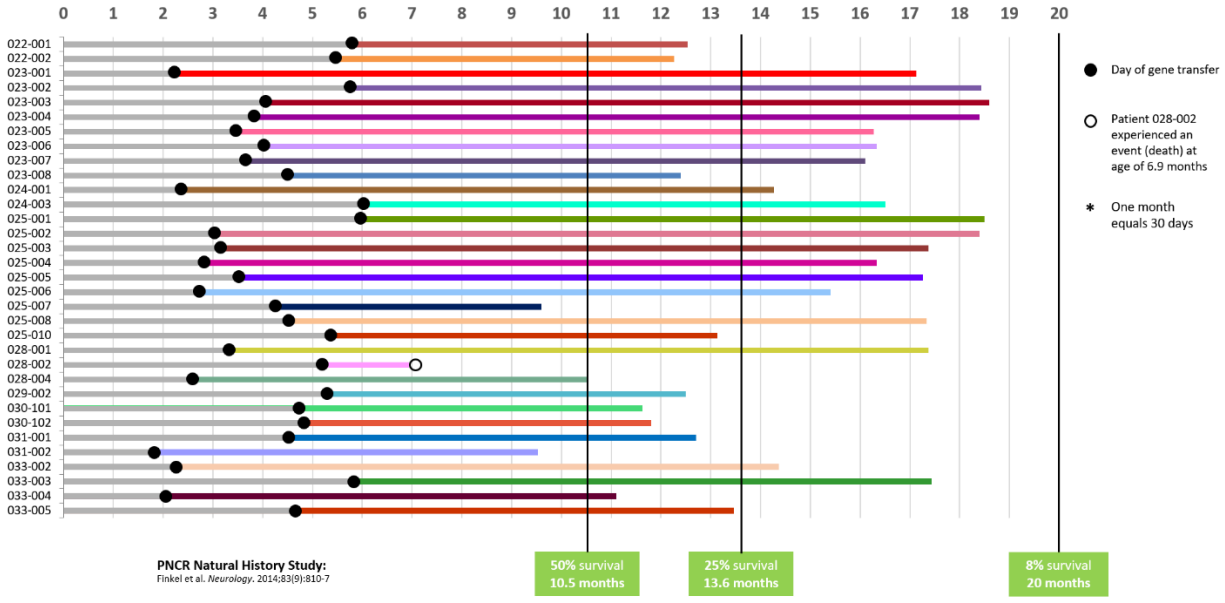


Figure 9: Event Free Survival in Study CL-302 (31 Dec 2019 data cut off)

Motor milestone achievement

As shown in Table 21, as of the 31 Dec 2019 data cut-off, 8 patients met the milestone (Bayley Scales Gross Motor Subset item #26), "sitting alone without support for at least 30 seconds" (Bayley scales gross motor subset item #26), which constitutes functional independent sitting. The primary efficacy endpoint "independent sitting for at least 10 seconds at any time up to 18 months of age (WHO definition) was met by 6 of the 32 patients (18.8%)

Table 15: Video Confirmed Developmental Milestones (ITT Population) Study CL-302 (31 DEC 2019 data cut-off)

Milestone achieved	n/N	%
Holds head erect for at least 3 seconds without support	20/33	62.5%
Rolls from back to sides	8/32	25.0%
Sits independently without support for at least 30 seconds	8/32	25%
Stands with assistance	1/32	3.1%

CHOP-INTEND

Mean baseline CHOP-INTEND score was 27.5 (range 14, 55) with a median of 27.5. Thus, baseline CHOP-INTEND scores in CL-302 study patients were lower compared to CL-303 study patients.

The mean increases (improvement) from baseline to Month 1 (n=31), Month 3 (n=29), and Month 6 (n=27) after dosing were 5.9 (5.48), 10.1 (6.32), and 13.3 (6.54) points, respectively.

At of the 31 Dec 2019 data cut-off, 21 patients (63.6%) had scored ≥ 40 on the CHOP INTEND, and 12 patients (36.4%) had scored ≥ 50 and 1 patients had scored ≥60.

Bayley Scales of Infant and Toddler Development

As of the 31 Dec 2019 data cut-off 30 patients (93.8%) had ≥ 3 months of Bayley Scales data (range of 1 to 15 months of data). Improvement in performance was observed in both the Bayley Scales gross motor and fine motor subsets raw scores. In the gross motor subtest patients improved their mean (SD) raw score by 6.0 (7.16) points at Month 6 post-dose (n = 29), and by 9.6 (6.64) points at Month 12 (n=16). In the fine motor subtest patients improved their mean (SD) raw score by 19.2 (5.79) points at Month 6 post-dose (n = 29), and by 22.1 (4.18) points at Month 12 (n=16).

Study CL-304

Study CL-304 is an ongoing multi-centre, global, Phase III, open label, single arm study to assess the Safety and Efficacy of a single IV infusion of 1.1E14 vg/kg (equivalent to therapeutic dose cohort 2 CL-101). The target patient population is pre-symptomatic patients expected to develop SMA, bi-allelic deletion of *SMN1* and 2,3 or 4 copies of *SMN2* without the genetic modifier (c.895G>C) (ITT population). Patient with genetic modifier of *SMN1* point mutations are not excluded.

The protocol for Study CL-304 was modified in September 2018 after results of Good Laboratory Practice compliant 3-month mouse toxicology studies became available. As a result of these toxicology findings (arterial thrombosis), AveXis suspended enrolment of pre-symptomatic patients with 4 copies of *SMN2* in Study CL304. No patients with 4 copies of *SMN2* had been enrolled in Study 304 at the time of this protocol amendment by purpose, but one patient enrolled into cohort 2 was found to have four copies of *SMN2.* Therefore, cohort 3 includes one patient.

Study 304 will enrol ≥ 27 patients, including at least 15 patients with 2 copies of *SMN2* who meet the ITT criteria, and at least 12 patients with 3 copies of *SMN2* who meet the ITT criteria. Patients with *SMN1* point mutations or the *SMN2* gene modifier mutation (c.859G>C) may be enrolled but will not be included in the efficacy analysis sets.

The endpoints per cohort are:
Cohort 1 (2 *SMN2* copies): proportion of patients achieving milestone of independent sitting for at least 30 seconds at any visit up to 18 months of age.
Cohort 2 (3 SMN2 copies): proportion of patients achieving the ability to stand without support for at least 3 seconds at any visit up to 24 months of age.

Results

As of the 31 DEC 2019 data cut-off, 14 patients were enrolled and treated in Study 304 within cohort 1 (2 *SMN2* copies). Fifteen (15) patients were enrolled and treated in cohort 2.

Survival

As of the efficacy data cut-off date of 31 DEC 2019, patients in cohort 1 had been in the study for an average of 10.5 months (range: 5.1-18 months). Patients in cohort 2 had been in the study for an average of 8.74 months (range: 2-13.9 months). All patients in the study were alive and free of permanent ventilation at the data cut-off.

Motor milestone achievement

The motor milestone achievement during the study for cohort 1 is shown in Table 22. The motor milestone achievement for cohort 2 is shown in Table 23

Table 16: Highest achieved Video Confirmed Developmental Milestones (ITT Population) Study CL-304 Cohort 1 (2 copies of *SMN2)* (31 DEC 2019 data cut-off).

Milestone achieved	**n/N**
Holds head erect for at least 3 seconds without support[a]	14/14
Turns from back to both right and left sides	8/14
Sits alone without support for at least 30 seconds	8/14
Supports own weight for at least 2 seconds.	5/14
Walks with assistance	5/14
Walks alone[b]	4/14

[a]two patients had had control at screening. [b]patients achieving this milestone also met previous milestones

Table 17: Highest achieved Video Confirmed Developmental Milestones (ITT Population) Study CL-304 Cohort 2 (3 copies of *SMN2)* (31 DEC 2019 data cut-off).

Milestone achieved	**n/N**
Holds head erect for at least 3 seconds without support[a]	15/15
Turns from back to both right and left sides	9/15
Sits alone without support for at least 30 seconds	10/15
Stands with assistance	9/15
Pulls to stand	4/15
Walks with assistance	7/15
Walks alone[b]	3/15

[a]five patients had had control at screening. [b]patients achieving this milestone also met previous milestones

Bayley Scales of Infant and Toddler Development

In the gross motor subtest, 2-copy *SMN2* patients improved their mean (SD) raw score by 0.9 (1.94) points at Month 1 post-dose (n = 14), by 16.6 (4.37) points at Month 6 (n=8), by 29.3 (10.84) points at Month 12 (n = 4), and by 33.5 (9.40) points at Month 15 (n = 4); In the fine motor subtest, 2-copy *SMN2* patients improved their mean (SD) raw score by 1.5 (2.18) points at Month 1 post-dose (n = 13), by 16.4 (2.50) points at Month 6 (n = 8) , by 27.3 (2.63) at Month 12 (n=4) and by 30.5 (0.58) at Month 15 (n=4) Figure 10.

The scaled score reflects performance according to age as compared with other, normally developing children of the same age. The mean score is 10, with standard deviation of ± 3 points; thus, approximately 97% of children tested will fall within 2 standard deviations of the mean (scores 4-16). In the 2-copy *SMN2* cohort, 7 patients (of 14; 50%) have Bayley gross motor subtest scaled scores within 2 SD of the mean for age (i.e., scaled score ≥ 4) at their most recent visit prior to the data cutoff. All (14 of 14; 100%) patients in the 2-copy *SMN2* cohort have Bayley fine motor scaled scores within 2 SD of the mean for age.

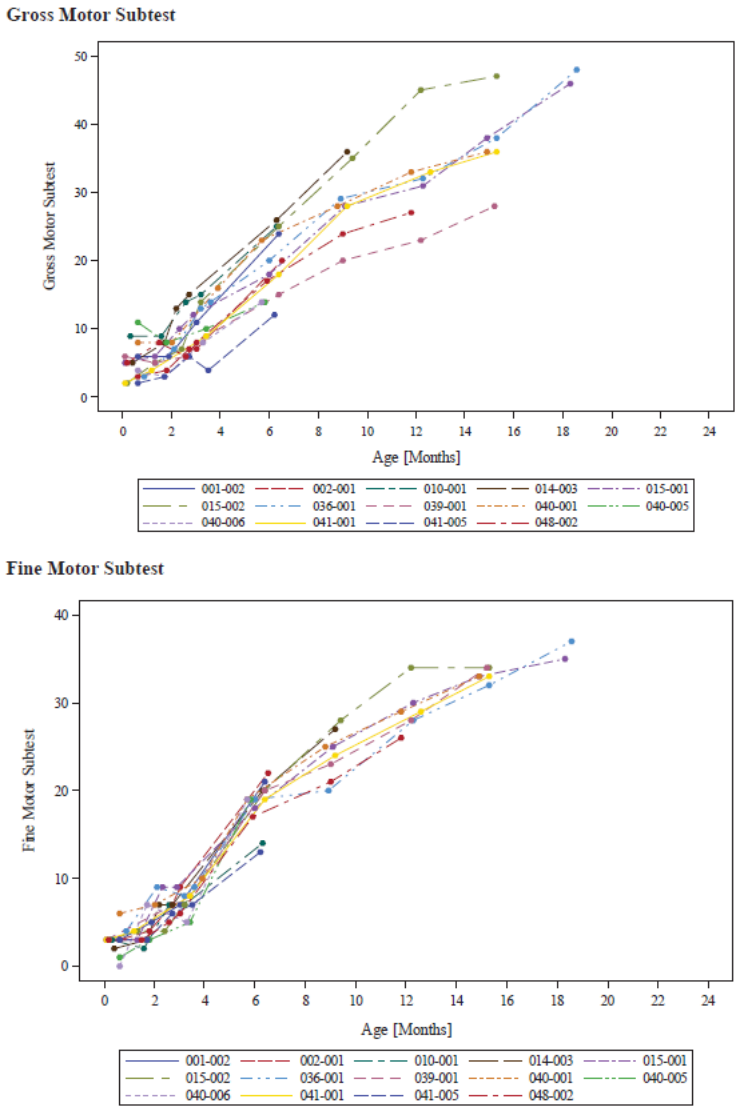


Figure 10 Bayley Scales Gross and Fine Motor Subtests (Raw Scores) in Patients with 2 Copies of SMN2 (cohort 1, 31 DEC 2019 Data Cut-off)

In cohort 2 (3 SMN2 copies) all patients (100%) had at least 2 months of Bayley Scales data (range of 1 to 14 months of data). In the fine motor subtest, 3-copy *SMN2* patients improved their mean (SD) raw score by 1.6 (1.94) points at Month 1 post-dose (n = 13), by 16.5 (2.81) points at Month 6 (n=6), and by 24.3 (3.59) at Month 11 (n=4). In the gross motor subtest, 3-copy *SMN2* patients improved their mean (SD) raw score by 2.6 (2.26) points at Month 1 post-dose (n = 13), by 17.5 (4.42) points at Month 6 (n=6), and by 32.0 (2.31) at Month 11 (n=4).

All 15 patients have Bayley gross motor subtest scaled scores within 2 SD of the mean for age at their most recent visit prior to the data cut-off. Fourteen (14; of 15; 93.3%) patients in the 3-copy *SMN2* cohort have Bayley fine motor scaled scores within 2 SD of the mean for age at their most recent visit prior to the 31 Dec 2019 data cut-off.

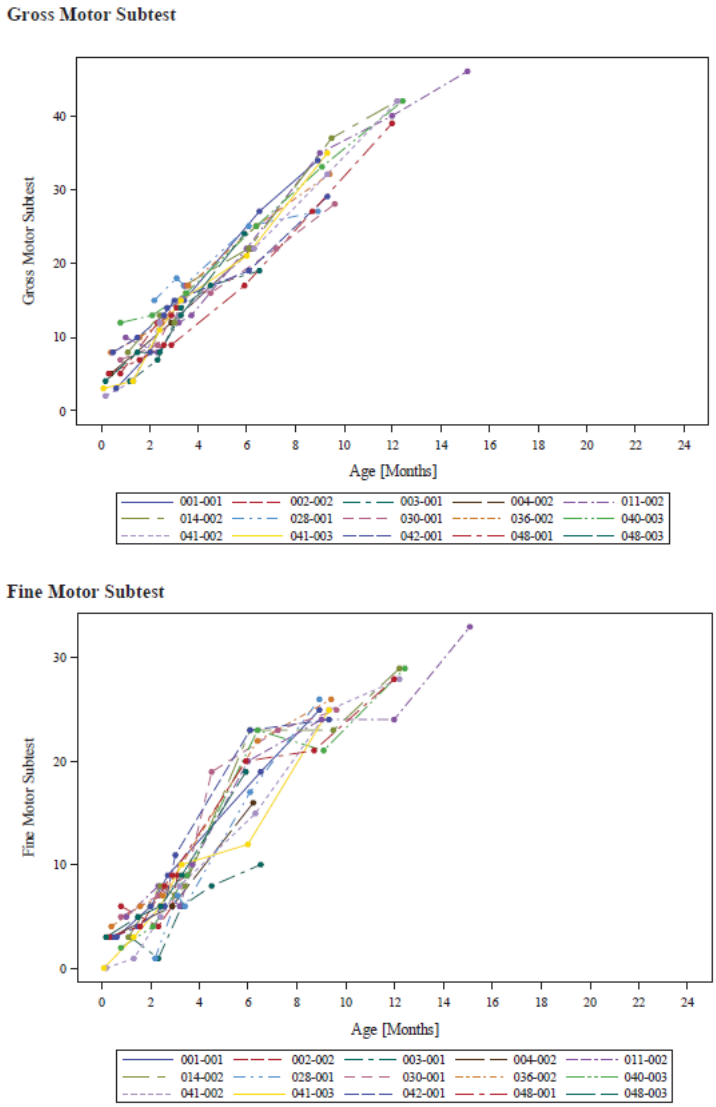


Figure 11: Bayley Scales Gross and Fine Motor Subtests (Raw Scores) in Patients with 3 Copies of SMN2 (cohort 2, 31 DEC 2019 Data Cut-off)

Analysis performed across trials (pooled analyses and meta-analysis)

As of the 31 Dec 2019, a total of 81 patients with 2 copies of *SMN2* have received Zolgensma in the above listed trials, referred to as "the 2-copy *SMN2* pool". The mean (±SD) follow-up time (time since receipt of Zolgensma) was 14.21 (±5.48) months, with a range of 1.8 to 25.7 months.

Kaplan-Meier analysis of survival data was performed for the 2-copy *SMN2* pool versus historical, untreated controls from the NeuroNEXT and PNCR studies. The survival curve of the 2-copy *SMN2* pool was statistically significantly different from the curves generated from the two historical control studies (log-rank test, P<0.0001; Figure 12). Therefore, the survival of 2-copy *SMN2* individuals from Zolgensma studies is superior to the survival of historical, untreated controls.

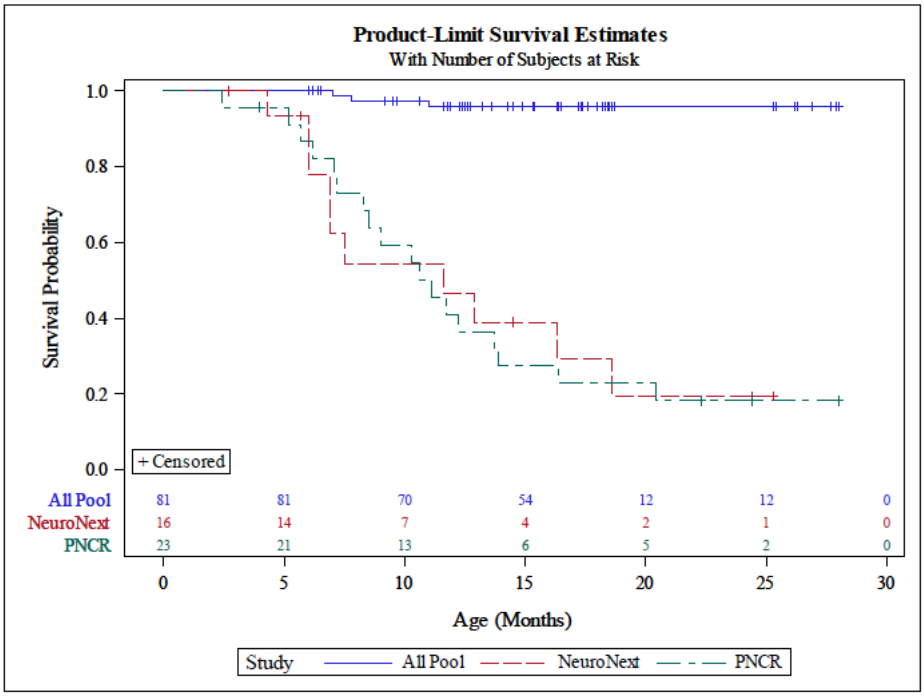
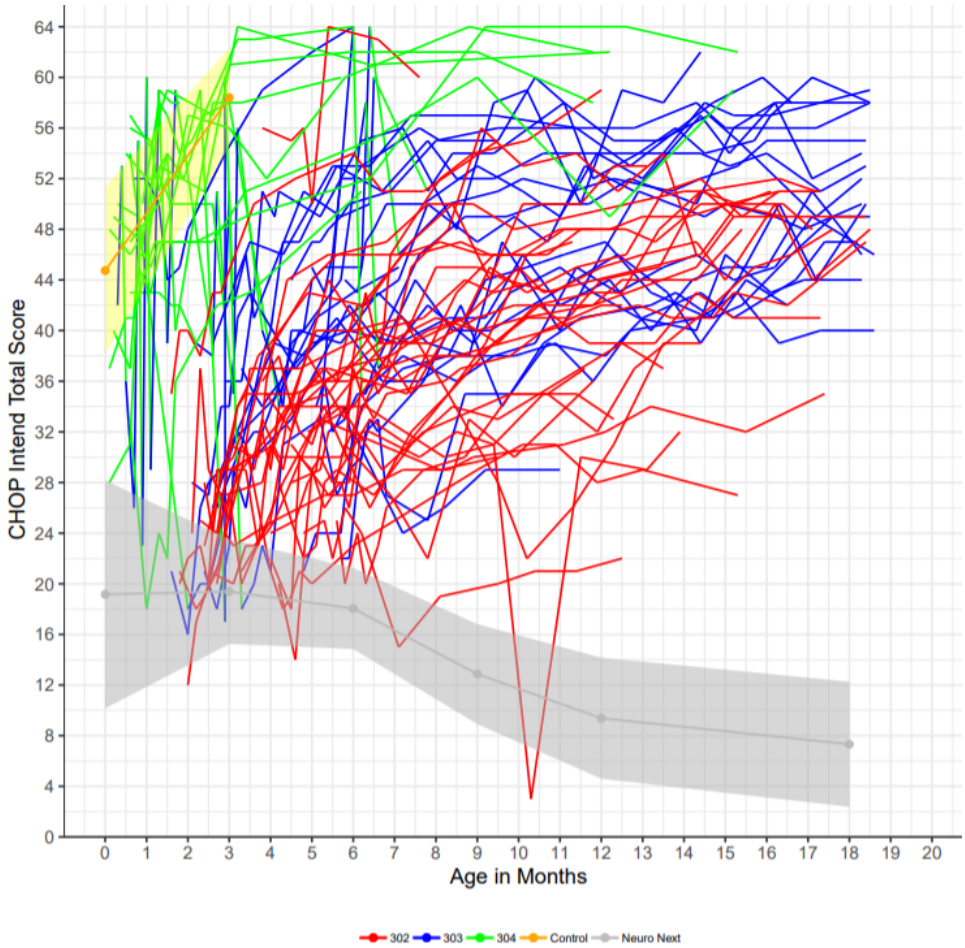


Figure 12: Kaplan-Meier Survival Curves of 2-Copy *SMN2* Pool, NeuroNEXT, and PNCR Data

Next, developmental milestone achievement was assessed for the 2-copy *SMN2* pool, and the total number of Zolgensma-treated individuals who achieved each developmental milestone at least once was tabulated. Not all milestones were assessed in all studies, but all milestones assessed were confirmed by central video review. The Bayley Scales milestones achieved by the largest to smallest proportion of the 2-copy *SMN2* pool are: head control (58 of 81; 71.6% achieved), sits without support (39 of 81; 48.1% achieved), rolls from back to sides (38 of 81; 46.9% achieved), stands with assistance (7 of 69; 10.1%), walks with assistance (8 f 81; 9.9%), stands alone (7 of 81; 8.6%), walks alone (5 of 81, 6.2%), pulls to stand (4 of 69; 5.8%), and crawls (3 of 69; 4.3%). The WHO milestones achieved by the largest to smallest proportion of the 2-copy *SMN2* pool are: sitting without support (34 of 81; 42%), standing with assistance (7 of 81; 8.6%), walking alone (4 of 69; 5.8%), walking with assistance (4 of 69; 5.8%), and standing alone and hands-and-knees crawling (both 3 of 69; 4.3%).

CHOP-INTEND scores in the 2-copy SMN2 pool rapidly improved (Figure 13) by a mean (±SD) of +6.5 (±5.91) at 1 month post-dose, +11.8 (±6.93) at 3 months post-dose, +15.4 (±8.14) at 6 months post-dose, and +19.9 (±8.54) at 12 months post-dose. The mean (±SD) CHOP-INTEND score at the most recent visit prior to the data cutoff was 49.8 (±10.54) for the 2-copy *SMN2* pool participants.



Source: Study CL-302, Study CL-303, and Study CL-304
Figure 13 CHOP-INTEND Total Score – Pooled Analysis

Ventilator use was monitored in the 2-copy *SMN2* pooled studies. Overall, 11 of 81 (13.5%) of 2-copy *SMN2* patients required ventilator support at baseline. Ten of these patients also used ventilator support during the study. There were another 23 patients who did not need ventilator support at baseline, but who eventually used ventilator support during the study. Therefore, a total of 33 of 81 (40.7%) patients used ventilator support during the study. A total of 48 of 81 (59.3%) 2-copy *SMN2* patients remained free of ventilator support during the studies.

Feeding support was monitored in the 2-copy *SMN2* pooled studies, also. Nineteen (19) of 81 patients (23.5%) used feeding support during the study. This included 15 patients who had feeding support at baseline. Of the remaining 66 patients, 11 (16.7%) needed feeding support during the study after receiving Zolgensma. A total of 62 of 81 (76.5%) 2-copy *SMN2* patients remained free of feeding support during the studies.

Clinical studies in special populations

No clinical studies were conducted in special populations.

2.5.4. Discussion on clinical efficacy

In the dossier, 6 studies are included (CL-101, LT-001, CL-303, CL-302 and CL-304, and one patient from study CL-306) in support of the indication 5q SMA with up to 3 copies of *SMN2*.

CL-303 is considered pivotal for the use of the product in symptomatic patients with 5q SMA and 2 *SMN2* copies.

The preliminary data from CL-302 and CL-304 is considered supportive for the use of the product in 5q SMA patients 2 copies of *SMN2* (CL-302) and for the use of Zolgensma in pre-symptomatic patients with 2 or 3 *SMN2* copies (CL-304).

Study CL-306 is a phase 3 trial investigating Zolgensma in 5q SMA Type 1 patients in Japan, South Korea, and Taiwan. So far, only one patient has been enrolled and treated. This study is not discussed here.

Data from the ongoing study CL-102 was included in the dossier, but the efficacy is not discussed either as it is an ongoing study to investigate the intrathecal route of administration in sitting but non-ambulatory patients (presumed SMA Type 2).

Design and conduct of clinical studies

As of 31 Dec 2019 the clinical program (including CL-101 cohort 2, CL-302 and CL-303 and CL-304 cohort 1 and 2) includes 98 subjects treated with the proposed IV dose I, i.e. 12 in cohort 2 of CL-101 (process A), 22 in CL-303, 33 in CL-302 and 30 in CL-304.

Considering only process B, the sample size is 86 for which efficacy data has been provided. This is substantial given the incidence of the disease, however, more information both in number of patients as in follow-up time is necessary to assess the maintenance of the treatment and to assess the clinical relevance of the observed effects (especially related to milestones achievement). This data will be obtained from the long term follow up studies LT-001 and LT-002 and the RESTORE registry study.

Dose finding

No dedicated dose finding/ dose response studies were conducted for Zolgensma. Justification of the proposed therapeutic dose of 1.1E14 vg/kg is based on the non-clinical studies and a single ascending dose study i.e. study CL-101.

Main study: Study CL-303

This is a Phase 3, open-label, single-arm, single-dose study of Zolgensma (gene replacement therapy) that enrolled 22 patients with SMA Type 1 who were either symptomatic or pre-symptomatic with no functional *SMN1* gene as well as 1 or 2 copies of *SMN2* and who are < 6 months (< 180 days) of age at the time of gene replacement therapy.

The study population is supportive of part of the proposed indication (patients with 2 *SMN2* copies). The definition of SMA type 1 patients is in accordance with international consensus guidelines[10].

Co-Primary endpoints are survival at 14 months of age and the proportion of patients that achieve functional independent sitting for at least 30 seconds at the 18 months of age study visit. This is a widely recognized and clinically meaningful endpoint which has been agreed on in scientific advice/protocol assistance for Zolgensma.

The medicinal product used in this study was manufactured by process B.

In general the historical controls are considered adequate for comparison with the study population. Although there are indications that the patients in the PNCR cohort has less severe disease (as expressed by the older age of the included patients), this is not considered a major issue since the potential bias this creates i.e. these patients are expected to experience the event earlier, is not in favour of

[10] Mercuri et al, Diagnosis and management of spinal muscular atrophy: Part 1: Recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. Neuromuscular disorders 2018.

Zolgensma. 'Ventilatory requirement prior to 6 months of age' is considered the most meaningful characteristic, as 21/23 of the PNCR and 22/22 of the CL-303 cohort did not require ventilator support prior to 6 months of age. Therefore, ventilator requirements prior to 6 months of age are considered of relevance for contextualisation of the ventilator requirements for determination of 'survival' in study CL-303.

Based on the literature available it can be concluded that few if any patients suffering from SMA type 1 survive the first 18 months of life without any ventilatory requirement and nutritional support. Further it can be concluded that few (effectively zero) patients suffering from SMA type 1 would be expected to be able to effectively swallow safely and maintain weight within normal growth parameters absent the provision of mechanical nutrition support through 18 months of age.

Supportive studies:

Study CL-101

Study Zolgensma-CL-101 was a Phase 1, non-randomised, open label, uncontrolled, single-centre, single-infusion, ascending-dose, study to evaluate the safety and efficacy of Zolgensma in up to 15 patients with Type 1 SMA. Outcomes were compared to the expected outcomes based on the two natural history cohorts derived from the PCNR and NeuroNext studies with respect to sex, race, ethnicity and CHOP-INTEND score.

SMA type 1 patients were included in this clinical study, defined by a bi-allelic mutation/deletion of *SMN1* and 2 copies of *SMN2*, together with an age of disease onset ≤6 months and clinical signs of hypotonia.

Patients were treated in two dose cohorts; a low dose of 6.7E13 vg/kg (cohort 1) and a higher dose of 2.0E14 vg/kg (cohort 2). The medicinal product used in this study was manufactured by process A.

The primary endpoint in this study was survival at 13.6 months of age, defined as time from birth to either death or permanent (≥16 hours per day) ventilatory support.

Key secondary endpoints were change from baseline in CHOP-INTEND score and motor milestone. The CHOP-INTEND score is a validated motor function score for SMA type 1 children. Although this score is considered clinically meaningful, this is not a norm-referenced scale which hampers the assessment of the development in relation to their healthy peers. Bayley scale assessments were initiated in patients reaching the top of the CHOP-INTEND. Since this is a norm-referenced scale this outcome is expected to provide clinically meaningful information.

Exploratory endpoints included maintaining the ability to thrive and the proportion of patients who were independent of ventilatory support.

Efficacy analyses were conducted at 3 time points, the first two time points corresponded with those of the major efficacy endpoints of the natural history study (13.6 and 20 months of age), and the third time point corresponded with at least two years follow up for all patients (24 months post-dose). The efficacy endpoint of time to death or permanent ventilation was compared to the natural history estimate using a 1-sample binomial test at the time patients were 13.6 or 20 months of age (corresponding to 25% and 8% survival in the natural history study.

The change from baseline in CHOP-INTEND score was analysed using MMRM. This is an efficient analysis in a small study population with multiple measurements per patients.

Study LT-001

Maintenance of efficacy is assessed annually in this study by using developmental milestones, overall survival, and the requirement for ventilation support are specifically assessed annually. Although important mile stones of motor development are captured (WHO score), this is not considered sufficient

to address long term efficacy. Bayley scale assessments has been included in the long-term follow up studies. This information provides some insight in the full development of the growing child and is necessary to make an informed decision at the time of treatment.

Study CL-302

This study has been designed as the European counterpart for study CL-303. Enrolment criteria were less strict with regard to ventilatory support use allowing slightly worse patients to be included.

Achieving the motor milestone of independent sitting according to WHO criteria up to 18 months of age is reflected in the primary endpoint, while event-free survival is reflected in the key secondary endpoint. The study population is supportive for part of the proposed indication, namely symptomatic patients with 2 *SMN2* copies.

The medicinal product used for this study was manufactured by process B.

Study CL-304

Study CL-304 is a phase III, open-label, single arm study designed to assess the efficacy and safety of Zolgensma in pre-symptomatic patients with 2 (cohort 1), 3 (cohort 2) or 4 (cohort 3) copies of *SMN2*.

The study population is supportive of part of the proposed indication (pre-symptomatic patients with 2 or 3 *SMN2* copies).

The primary efficacy endpoint for cohort 1 is: Proportion of patients achieving the development milestone of functional independent sitting at any visit up to 18 months of age.

The primary efficacy endpoint for cohort 2 is: Proportion of patients achieving the ability to stand without support for at least 3 seconds at any visit up to 24 months of age.

The medicinal product used in this study was manufactured by process B.

Data from the PCNR is used to construct a natural history for both patient cohorts. In general, the historical controls are considered adequate for comparison with the study population of cohort 2 (2 *SMN2* copies) since this group is relatively homogeneous. The natural history of patients with 3 *SMN2* copies is very heterogeneous and more information from the available literature and the RESTORE registry is considered necessary to construct a reliable natural history for this patient population.

The inclusion of patients with 4 *SMN2* copies was stopped after dose related findings in the ventricles of the heart in a 3 month-long mouse toxicology study were reported. A No Adverse Effect Level was not identified for drug related heart findings in the mouse. Based on these findings it is considered justified that patients with mild disease (as patients with 4 copies of *SMN2* generally are) are excluded. Although this is regrettable for no information will be readily available for the type 3b patient the exclusion of the patients with 4 SMN2 copies is considered acceptable.

Efficacy data and additional analyses

Study CL-303

Twenty-six patients were screened in Study CL-303. Four patients (15.4%) failed screening and were not administered AVXS 101.

Of the patients enrolled in study CL-303, 19 patients completed the study; one patient is deceased, and two patients withdrew consent.

The available information supports that patients survive beyond the expected time (based on the natural history study). Due to the limited data and the heterogeneity of the observed responses, no prognostic factor can be identified. The applicant has committed to the development of a prognostic model for

expected treatment outcome taking into account data from all relevant patients and known covariant that might influence the treatment outcome.

All patients showed increase in CHOP-INTEND score from baseline during the study. The mean (SD) increases (improvement) from baseline to Month 1, Month 3, Month 6 and Month 12 after the administration of Zolgensma were 6.9 (5.35), 11.7 (6.40), 14.6 (7.04) and 16.4 (8.04) points, respectively.

CHOP-INTEND scores at 18 months of age (end of study visit) were available for 16 patients. The mean total score (SD) was 51.2 (5.67) and the mean change from baseline (SD) was 19.3 (9.13).

Twenty-one patients (95.5%) achieved a CHOP-INTEND score of ≥40, 14 (63,6%) achieved a score of ≥50 and 5 patients (22,7%) achieved a score ≥60 at any point of time in the study.

The analysis of the gross and fine motor subtests of the Bayley Scales of Infant and Toddler Development indicate an ongoing improvement for most patients with the better results seen for the fine motor development. From assessment of the individual data is becomes clear that most patients show improvements on both the fine and gross motor skills subsets of the Bayley's scale although the effect on the gross motor subset is less pronounced.

14 out of 22 patients had reached the milestone of independent sitting for ≥30 seconds. One patient was able to walk alone. No milestones were recorded for 3 out of 22 patients, these were the 3 patients not completing the study due to death and withdrawal from the study. This data is considered important for caregivers and physician and is adequately reflected in the SmPC.

18 of 22 patients (81.8%) were independent of ventilatory support (as assessed by Trilogy BiPAP data) at 18 months of age (co-secondary endpoint, p< 0.0001). In total, 15 of 22 patients (68.1%) did not require any non-invasive ventilatory support at any point during the study. Fifteen (15) of 22 patients (68.1%) received no non-oral feeding support at any time during the study. Seven patients (31.8%) received non-oral feeding support at some point during the study. This is a clear benefit compared to the expected nutritional and ventilatory support based on natural history data.

The ability to thrive at 18 months of age was a co-secondary endpoint defined as the ability to tolerate thin liquids, does not receive nutrition through mechanical support, and maintains weight consistent with age. Nine of 22 patients (40.9%) met the ability to thrive criteria at 18 months of age. This is significantly different with natural history and considered clinically relevant.

Supportive studies

Study CL-101

All patients treated in CL-101 were still alive at 13.6 and 20 months of age. At the end of the study, all patients were still alive with the exception on 1 patient in cohort 1 that transiently needed ventilatory support for >16 hours a day after a surgery.

All patients in cohort 2 showed an increased CHOP-INTEND score from baseline. Mean increases at months 1 and 3, were 9.8 and 15.4, respectively (n=12, both p<0.001). At Month 12, the mean increase from baseline was 25.7 (excluding patient E08-208).

At the end of the study (24 months post-dose), 11 patients from cohort 2 were able to hold their head erect without support for ≥ 3 seconds and sit with support, 9 patients were able to sit without support for ≥ 30 seconds, and 2 patients were able to walk alone.

Of the 7 patients included in the ability to thrive population, 2 patients lost the ability to thrive on oral nutrition in the 24 months follow up.

Non-invasive ventilatory support was used by almost all patients during the study.

Seven patients from cohort 2 did not receive enteral feeding prior to treatment. All these patients remained free of enteral feeding except for one needing nutritional support after a surgery. The 5 patients that received enteral feeding at baseline were able to feed orally at the end of the study.

The outcomes exceed the natural course of SMA type 1 that showed survival rates of about 25% at 13.6 months of age and 8% at 20 months of age. Patients in the natural history cohorts only show a decline in CHOP-INTEND score and never reach a score above 40 after 6 months of age. The milestone of independent sitting is never reached in the natural history cohorts. This is considered a large and clinically meaningful effect. It exceeds the natural course of SMA 1 on all these parameters many times.

Post-hoc analysis on CHOP-INTEND increases from baseline stratified by gender, age of dosing, baseline CHOP-INTEND, weight, baseline CMAP values and time interval between diagnosis and treatment were submitted. Given the small sample size, it is difficult to draw conclusions from this analysis.

The post-hoc analyses indicate that most benefit is likely achieved in young patients with high baseline motor function i.e. before the onset of irreversible motor neuron loss. Young patients with low motor function, or older patients with high motor function are also likely to benefit albeit to a lesser extent. This observation is adequately reflected in the current SmPC.

Study LT-001

Milestone achievements were recorded in study LT-001 to support persistence of efficacy. Nusinersen is documented as ongoing for 7 of the 13 enrolled patients (53.8%). This concomitant use of nusinersen hampers the assessment of the achievement of new milestones in these patients which can not solely be attributed to Zolgensma. In these cases, it is difficult to disentangle whether maintenance of effect or further improvement is due to Zolgensma, nusinersen or an additive effect.

As of 31 DEC 2019, no loss of milestones has been seen across the long-term follow-up cohort in Study LT-001. Moreover, new video-confirmed motor milestones of stands with assistance were achieved by 2 patients. Both of these patients have had a one-year follow-up visit and neither of these patients are reported to have used nusinersen at any point.

CL-304

Preliminary data from Study CL-304 was submitted by the applicant to support the safety and efficacy of Zolgensma.

Cohort 1 (2 *SMN2* copies):

As of the 31 DEC 2019 data cut-off, 14 patients were enrolled and treated in Study 304 within cohort 1. All had survived without invasive ventilation and were continuing in the study. The patients were in the study on average for 10.5 months (range 5.1-18 months).

Patients in this cohort show motor milestones development with is largely within the range for normal development. Four out of 14 patients have already achieved walking assisted and subsequently walking alone and 1 is walking with assistance.

Bayley scaled scores show motor function within 2 SD of indicating normal motor development.

Cohort 2 (3 *SMN2* copies):

As of the 31 DEC 2019 data cut-off, 15 patients were enrolled and treated in Study 304 within cohort 2. All had survived without invasive ventilation and were continuing in the study. The patients were in the study on average for 8.74 months (range 2-13.9 months).

Patients in this cohort show motor milestones development with is largely within the range for normal development. Three out of 15 patients have already achieved walking assisted and subsequently walking

alone and four were walking with assistance. Bayley scaled scores show motor function within 2 SD of indicating normal motor development.

The follow up time and the heterogeneity of the natural history make it not possible to draw conclusions on benefit at this moment. Completion of study CL-304 is one of the conditions for a conditional MA. In addition, the applicant has committed to further characterize the natural history from patients with 3 *SMN2* copies and SMA type 2/3a based on available literature and patients from the RESTORE registry.

CL-302

Updated data with data cutoff 31 DEC 2019 was provided for study CL-302.

Thirty-three patients of 30 planned patients have been treated. One patient (302-028-002) has died. The other 32 patients all survive without invasive ventilation and are continuing in the study, but one patient is not included in the ITT population for having been treated at 181 days of age (ITT population only includes patients treated up to 180 days of age).

In the ITT population, the patients ranged in age from 2.1 and 6 months at the time of treatment. Patients in Study CL-302 were older at the time of treatment compared to CL-303 study patients.

As of the 31 DEC 2019 data cut-off, 6 patients met the WHO-MGRS criteria for sitting without support for more than 10 seconds and 8 patients achieved the Bayley definition (Bayley Scales Gross Motor Subset item #26), "sitting alone without support for at least 30 seconds", which constitutes functional independent sitting.

Completion of study CL-302 is considered necessary to address the missing efficacy data in the context of a conditional MA.

In addition, more information both in number of patients as in follow-up time is necessary to assess the maintenance of the treatment and to assess the clinical relevance of the observed effects (especially related to milestones achievement). This data will be obtained from the long term follow up studies LT-001 and LT-002.

2.5.5. Conclusions on the clinical efficacy

The proposed indication is considered acceptable:

Zolgensma is indicated for the treatment of:

- patients with 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene and a clinical diagnosis of SMA type 1, or

- patients with 5q SMA with a bi-allelic mutation in the SMN1 gene and up to 3 copies of the SMN2 gene.

The efficacy of the commercial process B product in patients with 2 *SMN2* copies is based upon data from study CL-303 (pivotal study) and supported by preliminary data from study CL-302 and study CL-304. The survival and motor milestones achieved largely exceed the natural history of SMA type 1. Data from study CL-303 and preliminary results from study CL-302 and CL-304 are in line with the data from the process A product investigated in study CL-101. The expected benefit is directly related to the extent of functional motor neuron loss of a patient; however, the exact inverse correlation is yet unknown.

No data has been submitted to substantiate benefit in patients with 1 *SMN2* copies.

The data to substantiate benefit in patients with 3 *SMN2* copies is limited. Due to the heterogeneity in the natural history for these patients, it is unclear whether the effect exceeds the expected development.

It is considered that the efficacy for patients with 2 copies of SMN2 can be extrapolated to patients with 1 or 3 SMN2 copies (see benefit/risk assessment). The indication is therefore considered acceptable.

Including a genetic diagnosis in the indication is considered acceptable given that SMN2 copy number determination is part of the standard SMA diagnostic process (see discussion on pharmacodynamics).

The CAT considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients younger than 6 months of age with Spinal Muscular Atrophy Type 1 with One or Two *SMN2* Copies the MAH should submit final data on Study CL-303.

In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients younger than 6 months of age with Spinal Muscular Atrophy Type 1 with One or Two *SMN2* Copies the MAH should submit interim and final data on Study CL-302.

In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients to genetically diagnosed and pre-symptomatic patients equal or younger than 6 weeks of age at time of treatment with SMA with bi-allelic deletion of *SMN1* with 2 or 3 copies of *SMN2*, the MAH should submit interim and final data on Study Zolgensma-CL-304.

In addition, the CAT considers the following measures necessary to address issues related to efficacy:

In order to further characterise and contextualise the outcomes of patients with a diagnosis of SMA, including long-term safety and efficacy of Zolgensma, the MAH should conduct and submit the results of a prospective observational registry AVXS-101-RG001 according to an agreed protocol.

The applicant should also perform a further evaluation of the finished product specifications when primary and key secondary endpoint data from additional patients with 2 copies of SMN2 are available (i.e. completion of CL-302 and CL-304 cohort 1). Based on this evaluation, it should be determined whether tightening of the release specification limits is needed to improve consistency of the batches and ensure optimal clinical outcome.

The CHMP endorse the CAT conclusion on clinical efficacy as described above.

2.6. Clinical safety

Patient exposure

Table 25 shows the patients exposure of Zolgensma as of 31 December 2019. Nighty-eight (98) patients received the proposed therapeutic dose at the data cut-off of 31 December 2019. Study CL101 has been completed, with data available of 49->68 months of follow up after dosing (long term follow-up Study LT-001). Study CL-303 is also completed. Studies CL-302, CL-304, CL-306 and CL-102 are ongoing at the data cut-off. 32 Patients received Zolgensma by Intrathecal Treatment Administration in Study CL-102.

Study CL-306 is an ongoing Phase 3, open-label, single-arm study of a single, one-time dose of 1.1E14 vg/kg Zolgensma administered via IV infusion conducted in patients with SMA Type 1 who are genetically defined by a bi-allelic pathogenic mutation of the *SMN1* gene, with 1 or 2 copies of *SMN2*. At the time of data cut-off, one patient was included in the study. This patient will be summarized separately under 'adverse events'.

It is to be noted that the numbers of patients do not exactly align with the numbers of patients given in the efficacy section. The safety database also includes patients from IT-study CL-102, which are not described in the efficacy part. In addition, safety data of 43 patients who were treated in the US Managed Access Program (MAP) and post-marketing data up to 31 December 2019 is included in the safety update.

Table 18 Summary of Patient Exposure to Zolgensma by Route of Administration for Studies CL-101, CL-303, CL-102, CL-304, CL-302 and CL-306.

Route of Administration	**Intravenous**		**Intrathecal**		
Zolgensma[a] Dose	**6.7E13 vg/kg[b]**	**1.1E14 vg/kg**	**6.0E13 vg**	**1.2E14 vg**	**2.4E14 vg**
Number of Patients					
Study Number					
CL-101	3	12[c]	-	-	
CL-303	-	22[d]	-	-	
CL-102	-	-	3	25	4
CL-304	-	30[d,e]	-	-	
CL-302	-	33	-	-	
CL-306	-	1	-	-	-
Total Patients	**3**	**98**	**3**	**25**	**4**

[a] A single dose of Zolgensma was administered in all studies.
[b] Low dose Zolgensma (NCH product) administered to patients in Cohort 1 of Study CL-101 as directly measured by a validated ddPCR assay.
[c] Proposed therapeutic dose (NCH product) administered to patients in Cohort 2 of Study CL-101 (directly measured to be 1.1E14 vg/kg by a validated ddPCR method).
[d] Proposed therapeutic dose (AveXis product; equivalent to NCH product administered to patients in Cohort 2 of Study 101)
[e] one patient was enrolled in Study CL-304, Cohort 2 initially but was later removed from the cohort when the genetic re-confirmation showed that the patient had 4 copies of SMN2.

Adverse events

As of the data cutoff date of 31 December 2019, 101 patients received an IV infusion of Zolgensma in Studies CL-101, CL-303, CL-304, CL-302 or CL-306. The only patient who is included in study CL-306 is not included in the pooled safety data but will be described separately at the end of this section. Ninety-six (96) of the 97 patients (99%) experienced at least 1 TEAE and 56 patients (58%) were reported to have a Treatment Emergent Adverse Event (TEAE) considered by the investigator to be related to Zolgensma (Table 26). Forty-five (45) patients (46%) had at least one SAE and 39 patients (40%) had at least one TEAE that was Grade 3 severity or higher.

Two patients (2.7%), 1 in Study CL-303 and 1 in Study CL-302, were discontinued due to TEAEs that resulted in death. In Study CL-303 one patient withdrew from the study because of an TEAE of respiratory distress which was considered not related to treatment with Zolgensma.

Table 19: Summary of Numbers of Patients (%) for Treatment-Emergent Adverse Events by Study) for studies CL-101, CL-302, CL-303 and CL-304

	CL-101				CL-302		CL-303		CL-304			
	Low Dose N = 3		AVXS-101 N = 12		AVXS-101 N = 33		AVXS-101 N = 22		AVXS-101 N = 30		AVXS-101 Total N = 97	
Category	Subject (n)	%	Subject (n)	%	Subject (n)	%	Subject (n)	%	Subject (n)	%	Subject (n)	%
Patients With at Least One TEAE	3	100	12	100	32	97	22	100	30	100	96	99
TEAE of Grade III Severity or higher	3	100	10	83.3	13	39.4	10	45.5	6	20	39	40.2
TEAEs Related to Study Treatment	1	33.3	3	25	24	72.7	12	54.5	17	56.7	56	57.7
Serious TEAEs	3	100	10	83.3	19	57.6	10	45.5	6	20	45	46.4
TEAE Causing Study Discontinuation	0	0	0	0	1	3	2	9.1	0	0	3	3
TEAEs Resulting in Death	0	0	0	0	1	3	1	4.5	0	0	2	2

In Studies CL-101, CL-303, CL-304, and CL-302, 96 of 98 patients (85.3%) who had received the proposed therapeutic IV dose of Zolgensma had at least 1 TEAE. The most frequently reported TEAEs across Studies CL-101, CL-303, CL-304, and CL-302 (≥10.0%) were pyrexia (47 patients, 48.5%), upper respiratory tract infection (36 patients, 37.1%), vomiting (24 patients, 24.7%), constipation (22 patients, 22.7%), cough (20 patients, 20.6%), gastroesophageal reflux disease (17 patients, 17.5%), diarrhoea, (15 patients, 15.5%),pneumonia (15 patients, 15.5%), rash (14 patients, 14.4%), and transaminases increased (12 12.4%), nasal congestion and scoliosis (each 11 patients, 11.3%) and gastroenteritis (10 patients, 10.3%).

Related TEAEs are events that are considered possibly, probably, or definitely related to Zolgensma by the investigator.

At the proposed therapeutic IV dose, the most frequently reported TEAEs considered related to Zolgensma across Studies CL-101, CL-303, CL-304, and CL-302 (≥2 patients) were transaminases increased (12 patients, 12.4%) aspartate aminotransferase increased (9 patients, 9.3%), alanine aminotransferase increased, vomiting and hypertransaminasemia, thrombocytopenia (each 8 patients, 8.2%), pyrexia, gastroesophageal reflux disease and rash (each 4 patients, 4.1%), platelet count increased, troponin increased, gamma-glutamyltransferase increased and vascular disorder (each 3 patients, 3.1%), cushingoid, diarrhoea, constipation gastroenteritis, blood creatine phosphokinase-muscle/brain (MB) increased ,hepatic enzyme increased, liver function test increased, lymphocyte count decreased, platelet count decreased, feeding disorder and diastolic hypertension (each 2 patients, 2.1%).

Study CL-102

As of the data cutoff date of 31 December 2019, 31 of the 32 patients (96.9%) enrolled experienced at least 1 TEAE. Twelve (12) patients (37.5%) experienced TEAEs that were considered by the investigator to be related to Zolgensma. The most frequently events (≥2 patients) were hypertension (3 patients, 9.4%), lymphadenopathy, vomiting, pyrexia, aspartate aminotransferase increased (each in 2 patients 6.3%).

Study CL-306

One patient was included at time of the data cut-off of 31 December 2019. The patient was treated with Zolgensma at 5 weeks of age. The patient experienced the following AEs: respiratory distress, tachypnea, rhonchi and 2 episodes of fever. All events were mild, unrelated, and non-serious. No potential clinical

laboratory findings were reported. One vital sign met PCS parameters (heart rate >190bpm). This was transient and resolved without intervention.

Serious adverse events

At the proposed therapeutic dose in all studies, data cutoff of 31 December 2019, 45 patients (46.4%) had at least 1 SAE, and 39 patients (40.2%) had at least 1 TEAE that was Grade 3 severity or higher. Two patients (2%), one in Study CL-303 and one in Study CL-302 had a Grade 5 TEAE (fatal).

Pneumonia and upper respiratory tract infection, respiratory syncytial virus bronchiolitis were serious TEAE's events reported by more than 5% of patients (13.4% and 6.2%, respectively).

The incidence of Grade 3 and 4 TEAEs in Studies CL-101, CL-303, CL-304, and CL-302 is summarized by preferred term in Table 26.

Table 20 Serious Treatment-emergent Adverse Events (Safety Analysis Set) for Studies Zolgensma-CL-101, Zolgensma-CL-302, Zolgensma-CL-303, and Zolgensma-CL-304 Sorted by Overall Frequency of Primary MedDRA Term

	CL-101						CL-302			CL-303			CL-304					
	Low Dose N = 3			AVXS-101 N = 12			AVXS-101 N = 33			AVXS-101 N = 22			AVXS-101 N = 30			AVXS-101 Total N = 97		
System Organ Class Preferred Term	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%
Patients With at Least One Serious TEAE	7	3	100	53	10	83.3	50	19	57.6	38	10	45.5	6	6	20	147	45	46.4
Blood and lymphatic system disorders	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Thrombocytopenia	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Cardiac disorders	0	0	0	1	1	8.3	0	0	0	1	1	4.5	0	0	0	2	2	2.1
Cyanosis	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Tachycardia	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Gastrointestinal disorders	0	0	0	0	0	0	2	2	6.1	1	1	4.5	1	1	3.3	4	4	4.1
Duodenal ulcer	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Dysphagia	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Inguinal hernia	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3.3	1	1	1
Vomiting	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
General disorders and administration site conditions	0	0	0	0	0	0	2	2	6.1	0	0	0	0	0	0	2	2	2.1
Pyrexia	0	0	0	0	0	0	2	2	6.1	0	0	0	0	0	0	2	2	2.1
Hepatobiliary disorders	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Hypertransaminasaemia	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Infections and infestations	5	3	100	38	9	75	24	14	42.4	13	7	31.8	3	3	10	78	33	34
Adenovirus infection	0	0	0	2	2	16.7	0	0	0	0	0	0	0	0	0	2	2	2.1
Bacterial tracheitis	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Bronchiolitis	0	0	0	0	0	0	2	2	6.1	2	2	9.1	0	0	0	4	4	4.1
Croup infectious	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3.3	1	1	1
Device related infection	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Enterovirus infection	0	0	0	3	2	16.7	0	0	0	0	0	0	0	0	0	3	2	2.1
Exanthema subitum	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Gastroenteritis	0	0	0	1	1	8.3	2	2	6.1	0	0	0	0	0	0	3	3	3.1
Gastroenteritis viral	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1

	CL-101						CL-302			CL-303			CL-304					
	Low Dose N = 3			AVXS-101 N = 12			AVXS-101 N = 33			AVXS-101 N = 22			AVXS-101 N = 30			AVXS-101 Total N = 97		
System Organ Class Preferred Term	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%
Lower respiratory tract infection	0	0	0	2	1	8.3	1	1	3	0	0	0	0	0	0	3	2	2.1
Nasopharyngitis	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Parainfluenzae virus infection	1	1	33.3	2	2	16.7	0	0	0	0	0	0	0	0	0	2	2	2.1
Pharyngitis	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3.3	1	1	1
Pneumonia	0	0	0	10	7	58.3	7	4	12.1	2	2	9.1	0	0	0	19	13	13.4
Pneumonia bacterial	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Pneumonia parainfluenzae viral	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Pneumonia respiratory syncytial viral	1	1	33.3	2	2	16.7	0	0	0	0	0	0	0	0	0	2	2	2.1
Pneumonia viral	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Postoperative wound infection	0	0	0	2	1	8.3	0	0	0	0	0	0	0	0	0	2	1	1
Pyelonephritis	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3.3	1	1	1
Respiratory syncytial virus bronchiolitis	1	1	33.3	2	2	16.7	0	0	0	2	2	9.1	0	0	0	4	4	4.1
Respiratory syncytial virus infection	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Respiratory tract infection	0	0	0	0	0	0	3	3	9.1	0	0	0	0	0	0	3	3	3.1
Rhinitis	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Rhinovirus infection	0	0	0	5	2	16.7	1	1	3	2	1	4.5	0	0	0	8	4	4.1
Sepsis	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Upper respiratory tract infection	0	0	0	3	3	25	2	2	6.1	1	1	4.5	0	0	0	6	6	6.2
Urinary tract infection	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Viral infection	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Viral upper respiratory tract infection	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Injury, poisoning and procedural complications	0	0	0	2	2	16.7	0	0	0	0	0	0	0	0	0	2	2	2.1
Femur fracture	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Post procedural haemorrhage	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Investigations	1	1	33.3	6	4	33.3	4	2	6.1	4	3	13.6	0	0	0	14	9	9.3

	CL-101						CL-302			CL-303			CL-304					
	Low Dose N = 3			AVXS-101 N = 12			AVXS-101 N = 33			AVXS-101 N = 22			AVXS-101 N = 30			AVXS-101 Total N = 97		
System Organ Class Preferred Term	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%
Alanine aminotransferase increased	0	0	0	0	0	0	1	1	3	1	1	4.5	0	0	0	2	2	2.1
Aspartate aminotransferase increased	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Coagulation test abnormal	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Enterovirus test positive	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Human metapneumovirus test positive	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Human rhinovirus test positive	0	0	0	2	2	16.7	0	0	0	0	0	0	0	0	0	2	2	2.1
Norovirus test positive	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Oxygen saturation decreased	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Sleep study	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Transaminases increased	1	1	33.3	1	1	8.3	1	1	3	1	1	4.5	0	0	0	3	3	3.1
Metabolism and nutrition disorders	0	0	0	1	1	8.3	2	2	6.1	3	2	9.1	1	1	3.3	7	6	6.2
Abnormal weight gain	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Dehydration	0	0	0	1	1	8.3	0	0	0	0	0	0	0	0	0	1	1	1
Failure to thrive	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Feeding disorder	0	0	0	0	0	0	1	1	3	1	1	4.5	0	0	0	2	2	2.1
Hypercalcaemia	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3.3	1	1	1
Hypernatraemia	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Nervous system disorders	0	0	0	0	0	0	3	2	6.1	1	1	4.5	1	1	3.3	5	4	4.1
Hydrocephalus	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Hypoxic-ischaemic encephalopathy	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Lethargy	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3.3	1	1	1
Loss of consciousness	0	0	0	0	0	0	2	1	3	0	0	0	0	0	0	2	1	1
Product issues	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Device malfunction	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Respiratory, thoracic and mediastinal disorders	1	1	33.3	5	5	41.7	4	3	9.1	14	5	22.7	0	0	0	23	13	13.4

	CL-101						CL-302			CL-303			CL-304					
	Low Dose N = 3			AVXS-101 N = 12			AVXS-101 N = 33			AVXS-101 N = 22			AVXS-101 N = 30			AVXS-101 Total N = 97		
System Organ Class Preferred Term	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%	Event (n)	Subject (n)	%
Acute respiratory failure	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Atelectasis	0	0	0	1	1	8.3	0	0	0	2	1	4.5	0	0	0	3	2	2.1
Increased bronchial secretion	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
Pneumonia aspiration	0	0	0	2	2	16.7	0	0	0	1	1	4.5	0	0	0	3	3	3.1
Respiratory arrest	0	0	0	0	0	0	0	0	0	1	1	4.5	0	0	0	1	1	1
Respiratory distress	0	0	0	2	2	16.7	2	1	3	7	4	18.2	0	0	0	11	7	7.2
Respiratory failure	1	1	33.3	0	0	0	1	1	3	2	2	9.1	0	0	0	3	3	3.1
Surgical and medical procedures	0	0	0	0	0	0	4	2	6.1	0	0	0	0	0	0	4	2	2.1
Gastrostomy	0	0	0	0	0	0	3	2	6.1	0	0	0	0	0	0	3	2	2.1
Hospitalisation	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
UNCODED	0	0	0	0	0	0	3	3	9.1	0	0	0	0	0	0	3	3	3.1

Study LT-001

For Study LT-001, only SAE's and AESIs (gene therapy-related AEs; liver function enzyme elevations; new incidences of a malignancy or hematologic disorder, and new incidences or exacerbations of pre-existing neurologic or autoimmune disorders, and sensory abnormalities suggestive of ganglionopathy) are collected.

As of the data cutoff date of 31 December 2019, 8 of the 13 patients (61.5%) were reported to have at least 1 SAE. Reported SAEs have included events of pneumonia, respiratory distress, acute or chronic respiratory failure, bronchitis, cardiac arrest, gastroenteritis, hypoglycaemia, and dehydration. One patient in the low-dose group (Patient E-02-491) had an episode of hypoxemic respiratory failure due to a mucous plug leading to cardiac arrest, requiring resuscitation and intubation. The patient was subsequently extubated, returning to baseline status. This event was judged not to be related to Zolgensma treatment (data on file).

There were no adverse events of special interest (e.g., gene-therapy related AEs), including new occurrences of malignancies, new or worsening neurological disease, new rheumatologic or autoimmune illnesses, or late development of liver injury observed. In addition, no new safety signals were observed over a mean follow-up of 57 months. No patients discontinued because of AE's and no deaths were reported.

Laboratory findings

Haematology

Study CL-303 CL-304, and CL-302

In study CL-303, a transient decrease in platelets occurred in most patients after administration of Zolgensma at or around Day 7 and generally returned to baseline levels by Day 14. The baseline mean (±standard deviation [SD]) for platelets was 435.5 (±114.5) 109/L. The Day 7 mean (±SD) for platelets was 174.9 (±67.2) 109/L. Two patients had platelets below the lower limit of normal. The day 14 mean for (±SD) platelets was 394 (±112.5) 109/L. At the final visit, mean (±SD) platelet value was 315.8 (±110.6) 109/L.

The number (%) of patients meeting PCS criterion in haematology for the Safety Analysis Set of Study CL-303 is summarized in Table 27.

Table 21 Summary of Patients Meeting Criteria for Potentially Clinically Significant Haematology Values During the Treatment Period – Safety Analysis Set (Study CL-303

Parameter (unit) Criteria	Study CL-303 Overall (N=22)	
	n/N-obs	(%)
Eosinophils (%)		
Very high	5/22	(22.7)
Hemoglobin (g/L)		
Very low	0/22	--
Very high	3/22	(13.6)
Leukocytes (10^9/L)		
Very low	2/22	(9.1)
Very high	2/22	(9.1)
Lymphocytes (10^9/L)		
Very low	3/22	(13.6)
Very high	2/22	(9.1)
Neutrophils (10^9/L)		
Very low	14/22	(63.6)
Platelets (10^9/L)		
Very low	2/22	(9.1)

Note: N-obs = number of patients with at least one observation for the parameter being summarized.
Source: CL-303 Table 14.3.4.3.1.1

In Study CL-304, platelets of patients with 2 copies of SMN2 started at a baseline mean (±SD) of 401.77 (±123.68) 109/L. Platelets reached a mean (±SD) nadir of 265.10 (±79.881) 109/L on Day 7. Platelets recovered to a mean (±SD) value of 401.25 (±65.240) 109/L by Day 14. At Age 15 months, the last visit for which more than 2 observations were available, the mean (±SD) was 420.40 (±84.491) 109/L.

In patients with 3 copies of SMN2, the mean (±SD) baseline platelets were 342.40 (±163.26) 109/L. Platelets reached a mean (±SD) nadir of 269.33 (±124.54) 109/L on Day 7. Platelets recovered to a mean (±SD) value of 437.40 (±240.45) 109/L by Day 21. At Age 12 months, the last visit for which more than 2 observations were available, the mean (±SD) was 439.00 (±136.61) 109/L.

The number (%) of patients meeting PCS criterion in haematology for the Safety Analysis Set of Study CL-304 is summarized in Table 28.

Table 22 Summary of Patients Meeting Criteria for Potentially Clinically Significant Haematology Values During the Treatment Period – Safety Analysis Set (Study CL-304)

Parameter (unit) Criteria	Study CL-304 AVXS-101 1.1E14 vg/kg					
	SMN2 2 Copies (N=14)		*SMN2* 3 Copies (N=15)		*SMN2* 4 Copies (N=1)	
	n/N-obs	(%)	n/N-obs	(%)	n/N-obs	(%)
Eosinophils (%)						
Very low	0/14	--	0/15	--	0/1	--
Very high	1/14	7.1	1/15	6.7	0/1	--
Hemoglobin (g/L)						
Very low	0/14	--	0/14	--	0/1	--
Very high	1/14	7.1	0/14	--	0/1	--
Leukocytes (10^9/L)						
Very low	1/14	7.1	1/14	7.1	0/1	--
Very high	1/14	7.1	1/14	7.1	0/1	--
Lymphocytes (10^9/L)						
Very low	0/13	--	1/14	7.1	0/1	--
Very high	1/13	7.7	1/14	7.1	0/1	--
Neutrophils (10^9/L)						
Very low	3/14	21.4	3/14	21.4	0/1	--
Very high	0/14	--	0/14	--	0/1	--
Platelets (10^9/L)						
Very low	0/14	--	1/14	7.1	0/1	--
Very high	0/14	--	0/14	--	0/1	--

Note: N-obs = number of patients with at least one observation for the parameter being summarized.

In Study CL-302, platelets started at a baseline mean (±SD) of 286.2 (±115.8) 109/L. Platelets reached a mean (±SD) nadir of 187.1 (±74.5) 109/L at Day 7. Platelets recovered to a mean (±SD) of 493.0 (±149.2) 109/L by Day 21. At the Month 18 Visit the mean (±SD) was 330.5 (±3.54) 109/L.

The number (%) of patients meeting PCS criterion in haematology for the Safety Analysis Set of Study CL-302 is summarized in Table 29.

Table 23 Summary of Patients Meeting Criteria for Potentially Clinically Significant Haematology Values During the Treatment Period – Safety Analysis Set (Study CL-302)

Parameter (unit) Criteria	Study CL-302 Overall (N=33)	
	n/N-obs	(%)
Eosinophils (%)		
Very high	2/33	(6.1)
Hemoglobin (g/L)		
Very low	0/33	--
Very high	1/33	(3)
Leukocytes (10^9/L)		
Very low	0/33	--
Very high	8/33	(24.2)
Lymphocytes (10^9/L)		
Very low	1/33	(3)
Very high	5/33	(15.2)
Neutrophils (10^9/L)		
Very low	9/33	(27.3)
Platelets (10^9/L)		
Very low	4/33	(12.1)

Note: N-obs = number of patients with at least one observation for the parameter being summarized.
Source: CL-302 Table 14.3.4.3.1.1

Study CL-101

Decreases from baseline in platelet counts were observed at multiple time points; however, no clinically significant changes were noted, and all individual patient platelet counts were considered CTCAE Grade 1 during the study. Decreases were clinically asymptomatic, transient, and resolved without sequelae.

Study LT-001

As of the data cutoff of 31 December 2019, no TEAEs associated with haematology laboratory parameters were reported in Study LT-001.

Study CL-102

In study CL-102, a transient decrease in platelets occurred after administration of Zolgensma at or around Day 7 and generally returned to baseline levels by Day 14. The baseline mean (±SD) for platelets was 346.8 (±83.01) 109/L. The Day 7 mean for platelets was 270.1 (±108.67) 109/L. The Day 14 mean for platelets was 359.9 (±100.62) 109/L. At the final visit, mean platelet value was 344.7 (±89.36) 109/L.

Chemistry

Study CL-303

At the data cut-off of 31 December 2019, for Study CL-303, a transient increase in transaminases occurred in many patients who were administered Zolgensma during the first month after treatment. The baseline mean (±SD) ALT was 31.2 (±10.8) IU/L. The ALT maximum mean (±SD) was 189.5 (±558.19) IU/L and occurred on Day 7. The median ALT on Day 7 was 39. The mean (±SD) ALT on Day 14 was 39.5 (±61.2) IU/L. The median ALT on Day 14 was 24 IU/L. The Month 18 mean (±SD) ALT was 21.6 (±11.9) IU/L and the median ALT was 17 IU/L.

The baseline mean (±SD) AST was 40 (±9.7) IU/L. The AST maximum mean (±SD) was 227.5 (± 501.3) IU/L and occurred on Day 7. The median AST on Day 7 was 77. The Day 14 mean (±SD) AST was 43.6 (±11.8) IU/L. The median AST on Day 14 was 41.5 IU/L. The Month 18 mean (±SD) AST was 37.5 (±10.5) IU/L and the median was 36 IU/L. One patient had SAEs of AST increased (Grade 4, Day 7) and ALT increased (Grade 4, Day 7) and was considered to be probably related to Zolgensma and one patient had an SAE of transaminases increased (NR, Day 29) that was considered definitely related to Zolgensma. These events did not lead to patient discontinuation and resolved during the reporting period.

Hy's Law (39) criteria and liver function enzyme changes over time were assessed to determine if a signal related to drug induced liver injury (DILI) occurred in any individual or group of patients. None of the patients met the criteria for Hy's Law.

The number (%) of patients meeting potentially clinically significant criterion for the Safety Analysis Set of Study CL-303 is summarized in Table 30.

Table 24 Summary of Patients Meeting Criteria for Potentially Clinically Significant Chemistry Values During Treatment Period – Safety Analysis Set (Study CL-303)

Parameter (unit) Criteria	Study CL-303 Overall (N=22)	
	n/N-obs	(%)
Alanine aminotransferase (IU/L)		
Very High	4/22	(18.2)
Albumin (g/L)		
Very low	1/22	(4.5)
Alkaline phosphatase (IU/L)		
Very high	1/22	(4.5)
Aspartate aminotransferase		
Very high	5/22	(22.7)
Bilirubin (µmol/L)		
Very high	2/22	(9.1)
Creatine Kinase MB (µg/L)		
Very high	22/22	(100)
Creatinine (µmol/L)		
Very high	0/22	--
Gamma glutamyl transferase (IU/L)		
Very high	0/22	--
Potassium (mmol/L)		
Very high	3/22	(13.6)
Sodium (mmol/L)		
Very low	0/22	--
Very high	0/22	--
Urea nitrogen (mmol/L)		
Very high	0/22	--

MB = muscle and brain.
Note: N-obs = number of patients with at least one observation for the parameter being summarized.
Source: Study CL-303 Tables 14.3.4.3.2.1

Study CL-304

In Study CL-304 patients with 2 SMN2 copies, baseline mean (±SD) ALT was 21.14 (±11.85) IU/L. The maximum mean ALT (±SD) was 44.33 (±15.04) IU/L and occurred on Day 72. The ALT maximum value was 68 IU/L and occurred on Day 7. At Age 15 Months, the last visit for which more than 1 observation was recorded, ALT mean (±SD) was 27.20 (±8.59) IU/L.

In Study CL-304 patients with 2 SMN2 copies, baseline mean (±SD) AST was 42.43 (±22.27) IU/L. The maximum mean (±SD) AST was 65.33 (±12.42) IU/L and occurred on Day 72. The AST maximum value was 117 IU/L and occurred at Day 14. At Age 15 Months, the last visit for which more than 1 observation was recorded, AST mean (±SD) was 40.4 (±5.771) IU/L.

In Study CL-304 patients with 3 SMN2 copies, baseline mean ALT (±SD) was 20.60 (±9.59) IU/L. The maximum mean ALT (±SD) was 105.0 (± 139.58) IU/L and occurred Day 44. The maximum ALT value was 266 IU/L and occurred at Day 44. At Age 12 Months, the last visit for which more than 1 observation was recorded, ALT mean (±SD) was 22.20 (±9.63) IU/L.

In Study CL-304 patients with 3 SMN2 copies, baseline mean AST (±SD) was 35.07 (±14.53) IU/L. The maximum mean (±SD) AST was 116.33 (±144.35) IU/L and occurred at Day 44. The maximum AST value was 283 IU/L and occurred on Day 44. At Age 12 Months, the last visit for which more than 1 observation was recorded, AST mean (±SD) was 40.80 (± 4.324) IU/L.

The single Study CL-304 patient with 4 SMN2 copies had a baseline ALT of 13 IU/L, and a maximum ALT of 31 IU/L on Day 7. The ALT was 15 IU/L at the patient's last visit prior to the 31Dec2019 data cutoff, the Age 12 Months visit. This patient had a baseline AST of 24 IU/L and a maximum AST of 47 IU/L on Day 14. The AST was 27 IU/L at the patient's last visit prior to the 31Dec2019 data cutoff, the Age 12 Months visit.

Study CL-304 patients with 2 copies of *SMN2* did not meet very low PCS criteria for any chemistry parameter, see Table 31. The 2 copy *SMN2* patients in Study CL-304 met very high PCS criteria for alkaline phosphatase (1 of 13; 7.7%), bilirubin (5 of 14; 35.7%), creatine kinase MB (10 of 11; 90.9%), potassium (6 of 14; 42.9%), and troponin I (1 of 6; 16.7%) .

Study CL-304 patients with 3 copies of *SMN2* met very low PCS criteria for albumin (1 of 15; 6.7%) and met very high PCS criteria for alanine aminotransferase (2 of 15; 13.3%), alkaline phosphatase (2 of 15; 13.3%), aspartate aminotransferase (2 of 15; 13.3%), bilirubin (6 of 15; 40%), creatine kinase MB (2 of 10; 20%), potassium (6 of 14; 42.9%), and troponin I (2 of 8; 25%).

The single Study CL-304 patient with 4 copies of *SMN2* did not meet very low or very high PCS criteria for any chemistry parameter.

Table 25 Summary of Patients Meeting Criteria for Potentially Clinically Significant Chemistry Values During Treatment Period – Safety Analysis Set (Study Zolgensma-CL-304)

Parameter (unit) Criteria	Study CL-304 AVXS-101 1.1E14 vg/kg					
	SMN2 2 Copies (N=14)		*SMN2* 3 Copies (N=15)		*SMN2* 4 Copies (N=1)	
	n/N-obs	(%)	n/N-obs	(%)	n/N-obs	(%)
Alanine aminotransferase (IU/L)						
Very high	0/14	--	2/15	13.3	0/1	--
Albumin (g/L)						
Very low	0/14	--	1/15	6.7	0/1	--
Alkaline phosphatase (IU/L)						
Very high	1/13	7.7	2/15	13.3	0/1	--

Aspartate aminotransferase (IU/L)						
Very high	0/14	--	2/15	13.3	0/1	--
Bilirubin (µmol/L)						
Very high	5/14	35.7	6/15	40	0/1	--
Creatine kinase MB (ug/L)						
Very high	10/11	90.9	2/10	20	0/1	--
Creatinine (µmol/L)						
Very low	0/14	--	0/15	--	0/1	--
Gamma glutamyl transferase (IU/L)						
Very low	0/14	--	0/14	--	0/1	--
Potassium (mmol/L)						
Very high	6/14	42.9	6/14	42.9	0/1	--
Sodium (mmol/L)						
Very low	0/14	--	0/14	--	0/1	--
Very high	0/14	---	0/14	--	0/1	--
Troponin I (ug/L)						
Very high	1/6	16.7	2/8	25	0/0	--
Urea nitrogen (mmol/L)						
Very high	0/14	--	0/15	--	0/1	--

Note: N-obs = number of patients with at least one observation for the parameter being summarized.
Source: Study CL-304 Table 14.3.4.3.2.

Study CL-302

In Study CL-302 baseline mean ALT (±SD) was 33.4 (±11.7) IU/L. The maximum mean (±SD) ALT was of 65.5 (±7.8) and occurred at Month 18, but there are only 2 observations for that visit. For visits with more than 2 observations the maximum mean (±SD) ALT was 45.8 (±41.3) IU/L and occurred on Day 7. The maximum ALT value was 323 IU/L and occurred at Month 1. At Month 17, the last visit for which more than 2 observations were recorded, mean (±SD) ALT was 41.9 (±39.43) IU/L.

Baseline mean (±SD) AST was 46.7 (±14.3) IU/L. The maximum mean (±SD) AST was 106.8 (±129.3) IU/L and occurred on Day 7. The maximum AST value was 760 IU/L and occurred on Day 7. At Month 17, the last visit for which more than 2 observation were recorded, AST mean (±SD) was 49.9 (±25.30) IU/L.

The number (%) of patients meeting potentially clinically significant criterion for the Safety Analysis Set of Study CL-302 is summarized in Table 32.

Table 26 Summary of Patients Meeting Criteria for Potentially Clinically Significant Chemistry Values During Treatment Period – Safety Analysis Set (Study CL-302)

Parameter (unit) Criteria	Study CL-302 Overall (N=33)	
	n/N-obs	(%)
Alanine aminotransferase (IU/L)		
Very High	7/33	(21.2)
Albumin (g/L)		
Very low	0/33	--
Alkaline phosphatase (IU/L)		
Very high	2/33	(6.1)
Aspartate aminotransferase (IU/L)		
Very high	5/33	(15.2)
Bilirubin		
Very high	0/33	--
Creatine Kinase MB (µg/L)		
Very high	29/30	(96.7)
Creatinine (µmol/L)		
Very high	0/33	--
Gamma glutamyl transferase (IU/L)		
Very high	2/33	(6.1)
Potassium (mmol/L)		
Very high	1/33	(3)
Sodium (mmol/L)		
Very low	0/33	--
Very high	0/33	--
Troponin I		
Very high	2/6	(33.3)
Urea nitrogen (mmol/L)		
Very high	0/33	--

MB = muscle and brain.
Note: N-obs = number of patients with at least one observation for the parameter being summarized.
Source: Study CL-302 Tables 14.3.4.3.2.1

Study CL-101

In Study CL-101, 4 patients (26.7%) had a total of 5 TEAEs of elevated liver enzymes; 2 of these 5 events were classified as SAEs. Of the 4 patients with these events, 1 patient (the first patient treated under this protocol) was enrolled prior to the protocol amendment instituting administration of prednisolone. Following the protocol amendment to administer prednisolone 1 day prior to gene therapy only 3 of the remaining 14 patients receiving gene therapy had a TEAE of elevated liver enzymes. All of the elevated liver enzyme TEAEs were considered by the investigator to be definitely related to Zolgensma, were clinically asymptomatic (eg, no jaundice or symptoms of liver malfunction were reported in any of the patients), and transient, and resolved within the observation period with prednisolone treatment.

Study LT-001

As of the cutoff date of 31 December 2019, one patient enrolled experienced a TEAE associated with abnormal chemistry laboratory parameters. Onepatient had a TEAE of hypoglycaemia. This event started at Day 217 after treatment and was graded by the investigator as a CTCAE Grade 3 (severe) event that was unrelated to Zolgensma.

Study CL-102

In Study CL-102, baseline mean (±SD) ALT was of 21.9 (±7.7) IU/L. The maximum mean ALT (±SD) was 37.6 (±81.79) IU/L and occurred at Month 2. The ALT maximum value was 461 IU/L and occurred at Month 2. At Month 12, ALT mean (±SD) was 15.7 (±3.8) IU/L.

In Study CL-102, baseline mean (±SD) AST was of 40 (±10) IU/L. The maximum mean AST (±SD) was 46.8 (±37.6) IU/L and occurred at Month 2. The AST maximum value was 229 IU/L and occurred at Month 2. At Month 12, AST mean (±SD) was 31.7 (±6.2) IU/L. A single patient (Cohort 2, 1.2E14 vg) had very high values for ALT (≥1 to <3 years >150 U/L) and AST (≥1 to <3 years; >180 IU/L), along with hepatomegaly at Month 2 (11Oct2018). These ALT and AST increases were considered probably related to Zolgensma IT by the investigator.

Five patients had "very high" blood alkaline phosphatase (ALP) (≥1 to <3 years; >675 IU/L) that was not associated with hepatic enzyme abnormalities.

In none of the clinical studies the Hy's Law criteria were met.

Cardiac markers

Note to the reader: In the original protocols for the phase III studies only CK-MB was included. However, because of preclinical toxicology findings in the pivotal Good Laboratory Practice (GLP) compliant 3-month mouse toxicology studies, all study protocols were amended to incorporate additional cardiac safety monitoring. Troponin I was not collected initially in studies CL-302, CL-304, CL-102, or LT-001, but these protocols were amended to include measurement of troponin in patients enrolled under the amended protocol. As enrolment had completed in Study CL-303, the protocol was not amended to include measurement of troponin.

The cohort from study CL-304 with patients with 2 copies of SMN2 will be referred to as 'cohort 1' and the cohort with patients with 3 copies of SMN2 will be referred to as 'cohort 2'.

Treatment Emergent Adverse Events Table 33 (provided by assessor) shows the treatment emergent cardiac adverse events in cardiac disorder SOC and Investigations SOC.

Table 27 Summary of treatment emergent cardiac adverse events in cardiac disorder SOC and Investigations SOC – Safety Analysis Set

	CL-303	CL-302	CL-304	
System Organ Class Preferred Term	**N =22 n (%)**	**N =33 n (%)**	**Cohort 1 2 copies SMN2 N =14 n (%)**	**Cohort 2 3 copies SMN2 N =15* n (%)**
Cardiac Disorders				
Cyanosis	1 (4.5)	1 (3)	0 (0)	0 (0)
Bradycardia	0 (0)	1 (3)	0 (0)	0 (0)
Tachycardia	1 (4.5)	2 (6.1)	0 (0)	0 (0)
Left ventricular hypertrophy	0 (0)	0 (0)	1 (7.1)	0 (0)
Pericardial effusion	2 (9.1)	0 (0)	0 (0)	0 (0)
Investigations				
Blood pressure diastolic decreased	1 (4.5)	0 (0)	0 (0)	0 (0)
Blood pressure systolic increased	1 (4.5)	0 (0)	0 (0)	0 (0)
Blood creatine phosphokinase MB increased	2 (9.1)	0 (0)	1 (17.1)	0 (0)
Troponin increased	0 (0)	0 (0)	1 (7.1)	1 (6.7)
Cardiac murmur	1 (4.5)	0 (0)	0 (0)	0 (0)

*excluding the patient with 4 copies of SMN2.

Study CL-303

According to the Applicant, two cardiac-related TEAEs that were considered by the Investigator to be related to Zolgensma presented in one patient. The patient experienced one event of blood creatine phosphokinase MB increase of 24.1 µg/L (normal value < 5.1 µg/L, severity not reported) on Study Day 183 and one event of cardiac murmur (CTCAE grade 1) on Study Day 211, and one event of tachycardia (CTCAE grade 1) on Study Day 322. The cardiac murmur and tachycardia were considered by the Investigator to be unrelated to AVXS 101. The patient's elevated CK-MB was considered to be possibly related to AVXS 101 and was ongoing as of the 08 Mar 2019 data cut. One patient had a TEAE of blood pressure diastolic decreased on Study Day 1 that was considered to be possibly related to Zolgensma.

The other 5 events (cyanosis [CTCAE grade 1], tachycardia, [severity not reported], blood creatine phosphokinase MB increased [n=2, each CTCAE grade 1]), and blood pressure systolic increased [CTCAE grade 1], were not considered by the Investigator to be related to Zolgensma. The event of cyanosis (CTCAE grade 1) in a Patient (onset: Study day 43; resolution: Study day 44) was reported as a serious adverse event. No further serious treatment-emergent AEs were reported in Study CL-303 at the time of the data cutoff.

The baseline mean (±SD) CK-MB value was 12 µg/L (±7). Mean change from baseline in post-treatment CK-MB values ranged from –0.2 µg/L at Day 14 to +10.4 µg/L at Month 9. Individual patients change indicates no meaningful trends of changes in CK-MB values over time. The majority of patients in each study have high baseline values and show no shift from baseline to maximum or final values.

Study CL-302

Mean (±SD) CK-MB at baseline was 22.7 µg/L (±11.6). Mean (±SD) change from baseline in post-treatment CK-MB values ranged from –8.4 µg/L (±10.6) at Month 15 to 1.7 µg/L at Month 8 (single observation). Overall, mean (±SD) CK-MB ranged from 14.9 µg/L (±7.2) at Month 1 to a maximum of 23.5 µg/L (±10.1) at Month 6, with exception of Month 8, which had only one observation (7.4 µg/L).

A limited number of troponin I measurements were available for review (maximum of 5 observations at any time point). Mean (±SD) troponin I at baseline was 0.02 µg/L (±0.0, n=3). Mean (±SD) change from baseline in post-treatment troponin I values ranged from 0.0 µg/L (±0.0, n=4) at Month 6 to 0.027 µg/L (±0.023, n=3) at Month 1. The maximum mean troponin I value was 0.06 µg/L (±0.023, n=3) and occurred at the Month 2. At Month 6, the last measurement available, mean troponin (0.02 µg/L [±0.0, n=4]) was the same as at baseline.

Study CL-304 – cohort 1 (2 copies of SMN2)

As of the 31Dec2019 data cutoff patients with 2 copies of SMN2 had a mean (SD; number of observations) CK-MB at baseline of 17.21 µg/L (39.35; n=13). Mean change from baseline in post-treatment CK-MB values ranged from –0.45 µg/L on Day 14 to +12.70 µg /L on Day 7. For troponin I, mean troponin I at baseline and change from baseline were not available at the data cut-off of 31 December 2019. The mean (SD; number of observations) troponin I of patients with 2 copies of *SMN2* on Day 7 was 0.04 µg /L (no SD; n=1); on Day 30 it was 0.08 µg /L (0.064, n=3); at Age 6 months it was 0.03 µg/L (0.006, n=3); and at Ages 9 (n=3), 12, and 15 months (n=3) it was 0.02 µg/L (0.00).

Study CL-304 – cohort 2 (3 copies of SMN2)

Patients with 3 copies of SMN2 had a mean (SD; number of observations) CK-MB at baseline of 7.43 (3.35; n=8). Mean change from baseline in post-treatment CK-MB values ranged from –2.36 µg/L at Day 30 to +2.40 µg /L at Age 15 Months. For patients with 3 copies of SMN2, mean (SD; number of observations) troponin I at baseline was 0.06 µg/L (0.035; n=2). The change from baseline in post-treatment troponin I values for patients with 3 copies of SMN2 averaged (SD; number of observations) 0.03 µg/L (0.17, n=1) on Day 7 and 0.06 µg/L (0.02; n=2) on Day 30. Change from baseline was not available beyond Day 30. The mean (SD; number of observations) troponin I of patients with 3 copies of SMN2 was 0.12 µg/L (0.120; n=2) on Day 7; on Day 30 it was 0.06 µg/L (0.025; n=3); at Age 6 months it was 0.02 µg/L (0.00; n=4); at Age 9 months it was 0.02 µg/L (0.006; n=3); and at Age 12 months it was 0.02 µg/L (0.00; n=2).

The single patient with 4 copies of SMN2 had a baseline CK-MB of 4.80 µg /L. Mean change from baseline in post-treatment CK-MB values ranged from –1.80 µg /L at Day 7, Day 30, and Age 12 Months to –1.20 µg/L at Age 6 Months troponin I was not measured in this patient.

Study CL-101

All patients enrolled in Study CL-101 had elevated CK isoenzyme-MB (CK-MB) levels at baseline and at the majority of assessments during the study; however, none of the elevations in CK-MB were considered clinically significant by the study investigator. All observed elevations in CK-MB were clinically asymptomatic.

Minor elevations in cardiac troponin I levels were observed in Cohort 2 patients, particularly during the first 2 months after dosing. None of the elevations in cardiac troponin I observed during the study were considered clinically significant by the investigator. By the end of the study all values had either returned to within the normal range or no longer met the pre-defined criterion for clinical significance.

Study CL-102

Adult reference ranges are used for assessment of test results. Paediatric reference range for CK MB has not been established by the clinical laboratory. Twenty-nine (29) of the 30 patients had elevated values for CK-MB. Mean CK-MB was elevated at baseline and varied throughout the study. Cohort 2 (Zolgensma IT dose = 1.2E14 vg) had the most patient data (n=13). In Cohort 2, mean (± SD) CK-MB at baseline was 10.14 µg/L (±3.089), with a minimum of 6.6 µg/L and maximum of 15.4 µg/L. The CK MB mean at baseline exceeded PCS criteria (very high > 7.5 µg/L). Overall, mean (±SD) CK-MB ranged from 6.14 µg/L (±2.5) at Day 7 to a maximum of 14.34 µg/L (±8.4) at Month 2.

From all data provided it seems that individual patients change indicates no trends of changes in CK-MB values over time. The majority of patients in each study have high baseline values and show no shift from baseline to maximum or final values. As of the data cut off of 31 December 2019, troponin I data are limited data, however, troponin appears to increase within a few weeks after treatment and declines thereafter.

Electrocardiograms and echocardiograms

Selected ECG results were flagged when they met the pre-specified criteria:

Test/Measurement	Very Low (VL)	Very High (VH)
HR (bpm)	<70	>180
QTcB (msec)	--	>440
QTcF (msec)	--	>440

bpm = beats per minute; msec = milliseconds; QTcB = QT interval corrected for heart rate using Bazett's formula; QTcF = QT interval corrected for heart rate using Fridericia's formula.
[a] Bonafide 2013 (37).

For all clinical studies, no consistent electrocardiogram or echocardiogram findings were observed.

Deaths

Up to 31 December 2019, two deaths were reported. In study CL-303 a patient died five and a half month after receiving Zolgensma treatment at an age of 2 months. The months before the patient was hospitalised because of to poor weight gain and respiratory failure. Full naso-jejunal feeding and bi-level positive airway pressure (BiPAP), was introduced. The patient was discharged with resolution of both SAEs. Twenty five days later the child was found lifeless in her car seat after a 30-minute car ride. An increasing congestion for 2 days prior to this event was reported. The patient had not used the BiPAP consistently for the last week due to an ill-fitting mask.

At autopsy no concurrent explanation for death other than respiratory failure due to muscle weakness was found. There were no overt signs of infection. The scattered lymphoid follicles within the lungs are considered nonspecific reactive change compatible with subclinical viral infections or aspiration. The histological finding of increased intra-alveolar macrophages may also be seen in the setting of aspiration. The events and death were considered not related to Zolgensma according the Applicant.

In study CL-302, a 5-month-old male (weight 6.1 kg) was dosed with Zolgensma. Twelve (12) days post-dose the patient presented with respiratory distress and was subsequently intubated. The patient was found to have at least two viral respiratory infections. The condition deteriorated on Study Day 27 with significant hypotensive episodes followed by autonomic dysregulation, hypernatremia and seizures with MRI picture of diffuse widespread leukoencephalopathy and worsening of respiratory status with upper and lower left lobe collapse. Repeated attempts to extubate were unsuccessful and the patient died on Study Day 52 post-dose (25 January 2019).

At autopsy it was concluded that there was no specific post-mortem evidence of an inflammatory cause and that the encephalopathic clinical and pathological features appeared not directly related to the AAV gene therapy. The cause of death are severe complications due to respiratory tract infection, which in turn is quite common at Spinal Muscular Atrophy, resulting in hypoxic ischemic brain damage Biodistribution of both deceased patients shows that all analysed organs were targeted, with clear detection of Zolgensma vector genomes and RNA transcripts in all analysed organs. SMN protein expression was detected in all regions of the spinal cord, at levels that were nearly equivalent to non-SMA control spinal cord and in all regions of the one brain analysed. Motor neurons were similar in size and shape to the non-SMA control, unlike the loss and atrophied motor neurons in Non-Treated SMA Subjects.

Cases of special interest

Hydrocephalus clinical data

In study CL-303 one patient experienced a serious adverse event (SAE) of hydrocephalus on 1 Oct 2018. This SAE was determined to be possibly related to Zolgensma by the site investigator.

The patient was a 7-month-old boy with SMA type 1 (2 copy SMN2) diagnosed 3 May 2018. Prior to being dosed with Zolgensma his head circumference was above the 90th percentile for age per World Health Organization (WHO) growth charts. While pre- and post-dose MRIs are not available for all patients treated with Zolgensma, a brain MRI was performed at that time to evaluate the combination of delayed motor milestones and enlarged head size. The brain MRI was read as normal by the radiologist.

Subsequently he was treated with Zolgensma on 24 May 2018 at the age of 77 days. He presented to his neurologist 1 Oct 2018 (Study Day 131) with report of 4 days of intermittent squinting and a bulging fontanelle, along with 3 days of irritability, poor feeding, and emesis. Examination at that time was notable for macrocephaly, bulging fontanelle, intermittent left esotropia, and papilledema. A head ultrasound was performed, and it was notable for ventricular enlargement. He was admitted to the hospital on the same day for management of hydrocephalus.

A brain MRI on 2 Oct 2018 (Study Day 132) demonstrated ventriculomegaly consistent with communicating hydrocephalus. This was treated with a third ventriculostomy on 3 Oct 2018 (Study Day 133), followed by a ventriculoperitoneal shunt which was placed on 6 Oct 2018 (Study Day 136).

This patient experienced another severe adverse event (SAE) on 29 Oct 2018 (Study Day 159). At that time, he developed bilateral subdural fluid collections consistent with bilateral subdural hygromas due to cerebrospinal fluid leak from the ventriculoperitoneal shunt. He was hospitalized on 29 Oct 2018 (Study Day 159) for a surgical revision of the ventriculoperitoneal shunt. On 30 Oct 2018 (Study Day 160) surgery was performed. The patient had a right frontal omaya reservoir placed. A right occipital distal shunt revision was performed with a strata 2 valve programmed at level 2. The hospital course was complicated by a pseudomeningocele around the shunt that was treated with an ace wrap, which was used to apply light pressure. The event resolved on 02 November 2018 (Study Day 163) when the patient was discharged from the hospital. This SAE was assessed as Grade 2 in severity and unrelated to the investigational product by the principal investigator.

This patient experienced another SAE on 30 Jan 2019 (Study Day 252). At that time the patient was hospitalized for bronchiolitis and was intubated. During the hospitalisation another revision was made to the ventriculoperitoneal shunt to prevent future malfunction. Because the child was already hospitalized at the time of the shunt revision, this was not recorded as a SAE independent of the hospitalisation for bronchiolitis.

The patient withdrew consent due to an AE of respiratory distress (Grade 3) just before the end of study visit at 18 months of age. The AE of hydrocephalus was not recovered/not resolved at the time of discontinuation. This AE was initially reported as Grade 4 with a start date of 01 October 2018 and end date of 08 October 2018 (recovered/resolved with sequelae). The AE was downgraded to Grade 3 on 09 October 2019 with an end date of 05 March 2019 (recovered/resolved with sequelae). The event was then downgraded to Grade 2 on 05 March 2019 and was not recovered/not resolved at the time of discontinuation. This AE was considered to be possibly related by the investigator (all grades) and sponsor.

Hydrocephalus Post-marketing

In the post-marketing database (ARGUS), one case of hydrocephalus is reported by a physician (15 September 2019). This concerns a report of a 5-months old female who was enrolled in EAR – Individual Patient Request – Zolgensma intravenous – SMA – use as per USPI. She received Zolgensma on 27 Aug 2019 and nusinersen. Nusinersen start date, applied dose, frequency and route of administration are unknown. Her historical condition included hydrocephalus (Jun 2019). Current condition included congenital brain cyst. Information on concomitant medication was not provided. It was reported that the patient underwent a surgery due to congenital brain cyst causing the increase of cranial pressure (AE 'intracranial pressure increased'. The physician mentioned the patient had hydrocephalus and secondary to congenital brain cyst. After surgery, the patient was clinically well. The outcome of the event 'increased intracranial pressure' was not reported. The outcome of the event hydrocephalus was 'condition improving'. The event hydrocephalus was reported as serious (hospitalisation) by the physician. Seriousness of the event intracranial pressure increased was not reported. Seriousness assessment of the event intracranial pressure increased (medically significant) was upgraded based on the European Medicines Agency-Important Medical Event List. The causality of the event intracranial pressure increased was not reported. The event hydrocephalus was assessed as not related.

Acute Serious Liver Failure

Since 27 Sep 2018, a case of acute liver failure (SUSAR) was reported on 07 Mar 2019 in the US Managed Access Program with Zolgensma. A 6-month-old male concurrently receiving nusinersen with baseline elevations of AST and ALT (216 U/L and 234 U/L, respectively) developed acute liver failure approximately 51 days post Zolgensma dosing. Steroids were tapered starting Day 31 and ending Day 43. Liver biopsy revealed inflammation and fibrosis. The patient recovered with additional steroid therapy. Steroid therapy was ongoing as of 20 June 2019. At the time of approval, there was no updated information available on that case.

Liver biopsy:

- Massive ballooning degeneration of hepatocytes in Zone 3
- Massive mixed inflammatory infiltrate in the periportal areas (primarily CD8-positive T lymphocytes)
- Mild periportal collapse on reticulin stain, moderate periportal fibrosis with marked fibrosis of the central veins with mild inflammation and associated sinusoidal fibrosis with focal portal-central bridging.
- Marked bile ductular reaction with associated neutrophilic periductular inflammation.
- No adenovirus, CMV, HSV or EBV detected.

According to the Applicant, analysis of these clinical cases concluded that a systemic immune response was likely common to both cases. A case description is included in section 4.8 of the proposed SmPC. Moreover, a warning is included for careful consideration of treatment in patients hepatic impairment as well as monitoring of liver function for at least 3 months.

Pre-clinical Dorsal Root Ganglia Toxicity

In October 2019 the Applicant provided non-GLP toxicology results from a NHP study, indicating dorsal route ganglia might also be target organ of toxicity**.** In a completed toxicology study in cynomolgus macaques, '*most animals receiving intrathecal injection of Zolgensma developed minimal to marked dorsal root ganglia (DRG) mononuclear cell inflammation, sometimes accompanied by neuronal cell body degeneration or loss, at some or all examined levels (cervical to sacral). Inflammation was present at similar incidence and severity in animals given Zolgensma intrathecally alone or in combination with contrast agents.'*

However, the chosen route of administration for this non-GLP toxicology study was intrathecal. Although dorsal root ganglia are located only in proximity, excipients or impurities of Zolgensma, therefore, cannot be excluded per se as underlying reason for the observed findings. A uniqueness of the endothelial cells vascularizing the dorsal root ganglia is their large fenestrations and permeability to low and high molecular weight agents (Arvidson, 1979; Kiernan, 1996). Either being AAV9 related or Zolgensma formulation related such toxicity might also be seen after intravenous administration.

The autopsy plans as laid down in the clinical trial protocols include amongst others 'dorsal root' and 'DRG' as tissue sample for analysis of: vector presence, SMN gene expression, histology and immunohistochemistry. The autopsy report of the patient from study CL-302states dorsal root ganglia were retained for the trial investigators. RPT-952 and RPT-1342 indicate dorsal root ganglia of two deceased human patients treated intravenously with Zolgensma have been analysed.

For one deceased patient (study CL-303) an addendum to the original histopathology report is provided showing no findings indicative of dorsal root ganglion (DRG) toxicity, in particular no considerable myelin loss and no spheroids in the white matter funiculi. The dorsal and ventral roots that were available for examination appeared unremarkable.

For the other patient (study CL-302), an addendum to the original histopathology report and final immunostainings are also provided. The addendum describes the microscopic findings of the section containing the dorsal root ganglia (DRG) and related structures.

"Microscopy: Two blocks (B18 and B21) contain DRG. The DRG in block B18 has mostly unremarkable appearances, but there may be a few nodules of Nageotte. The DRG in block B21 shows ganglion cell loss and there is an excess of small round cells. On morphological grounds, the latter are mostly satellite cells and there are some nodules of Nageotte. In addition, there may be some chronic inflammatory cells. Immunohistochemistry is underway to identify the cell types.

Please see the description of the spinal cord in the main report. In particular, the dorsal columns show activation of microglia on CD68 staining but are unremarkable on the other stains (H&E, LFB, CD3 an neuroflilament).

Comment: The findings agree with those in the main report. In this addendum, I have expanded on the description of the findings in the DRG. There is some subjectivity in the assessment of DRG pathology but in my opinion, there are abnormalities in one of the DRG's. I have reviewed the case with my colleague, Dr. M., consultant neuropathologist, and he agrees with this assessment.

The cause of the abnormalities in the DRG is not certain, and I have considered three possible aetiologies.

1. Direct contribution of Spinal muscular atrophy to the pathology in the DRG.

2. Secondary to hypoxic/ischaemic injury in the terminal illness of this patients caused extensive acquired damage to the nervous system (including the DRG).

3. Secondary to treatment with Zolgensma.

Conclusion: Dorsal root ganglia: "Abnormal."

According to the pathologist it is not possible to distinguish the three possible causes on purely pathological grounds. It could be that more than one of these factors has contributed to the pathology in this case. However, it should be noted that DRG pathology is a feature of the underlying disease, and it may not be possible to determine with certainty if there was an additional contribution of Zolgensma treatment.

The additional immunohistochemistry has not determined the aetiology of the abnormalities in the DRG. While, there are some inflammatory cells identified, these are very limited in number. Summarizing, both histopathology and immunohistochemistry reports do not suggest a significant ongoing inflammatory process in the dorsal root ganglia.

Taken together, the histopathology reports of patients (study CL-303 and CL-302) provide some reassurance.

In addition, the Applicant provided a review of the current (non-)clinical literature of DRG toxicity associated with AAV vectors; results of non-clinical studies with AAV vectors; and a summary of the (non-)clinical data and post-marketing experience. The nonclinical finding of DRG inflammation reported in the non-human primate studies was not confirmed in clinical data thus far through the analysis of clinical data in either the Zolgensma IT or IV studies, as well as review of post-marketing safety data. Therefore, the clinical relevance of the DRG findings in non-human primate studies associated with IT administration of AAV vector gene therapies remains unknown.

A conclusion on Zolgensma related DRG toxicities at the current state of knowledge is hard to sustain. Both literature and this additional histopathology report discuss the underlying disease and severe exacerbations of accompanying complications as reasons. The best possible approach is intensive monitoring of the children for early and late symptoms.

For the long-term observational trial, the list of events of special interest (AESIs) has been revised by addition of sensory abnormalities suggestive of ganglionopathy next too hepatotoxicity, thrombocytopenia and cardiac adverse events. The Applicant amended the study protocols to include additional age appropriate sensory testing, and call for attention to new symptoms of pain, numbness, or paresthesias as part of the neurologic exam at baseline and at each visit in all ongoing clinical trial protocols for Zolgensma. The Applicant provided a clinical pathway to monitor for adverse events that might be indicative of DRG cell inflammation. Furthermore, DRG cell inflammation in included as an important potential risk of Zolgensma.

Study discontinuation

Two patients withdrew from study CL-303: the first patient is discussed below, for the second patient see under 'Cases of special interest'.

At the time of treatment, 16 May 2018, the patient was 154 days old, weighed 7.2 kg with a height of 70 centimetres. The patient had genetically-confirmed SMA Type 1 with bi-allelic deletion of SMN1, two copies of SMN2, and did not have the genetic modifier.

At the time of the data cutoff of 27 Sep 2018, the patient had experienced numerous TEAEs and with the exception of a TEAE of thrombocytopenia which began on Day 10 and resolved on Day 17, were otherwise considered unrelated to Zolgensma.

Of note, the patient had experienced serious adverse events (SAEs) of pneumonia, respiratory distress, bilateral atelectasis and severe dysphagia with date of onset of 20 Jul 2018. Ventilatory support was increased from continuous positive airway pressure (CPAP) to BiPAP due to increased work of breathing. With the exception of severe dysphagia, these TEAEs were considered resolved on 29 Jul 2018. The SAE

of dysphagia was considered unresolved. On 02 Oct 2018 the subject was admitted to the hospital for a G-tube placement and Nissen fundoplication. The surgery was uneventful, and the patient was transferred to the intensive care unit for extubation and recovery. On 03 Oct 2018 extubation was attempted, but failed with hypercarbic respiratory failure, and the patient was re-intubated. This was reported as SAEs of acute respiratory failure and atelectasis. On 09 Oct 2018 the patient was extubated and BiPAP continued. On 19 Oct 2018 the event of atelectasis was considered resolved. On 28 Oct 2018 the event of respiratory failure was considered resolved and the subject was discharged (data on file).

Other Clinical Programs

Eligible patients with a genetic diagnosis of SMA were treated in a Managed Access Program (MAP) under a Cohort Treatment Protocol. The program ended with approval of Zolgensma by the US FDA on 24 May 2019.

At the data cut-off of 31 December 2019, 43 patients, who received Zolgensma only, are included in the Managed Access Program (MAP) and/or RESTORE (registry) combined. The most frequently reported AEs include alanine aminotransferase and aspartate aminotransferases increases, each in 10 patients (23%); liver function test increased in 5 (11.6%) patients; thrombocytopenia and pneumonia, each in 4 (9%) patients; and constipation and vomiting, each in 3 (7%) patients. Although concomitant prednisolone use or dosage was commonly not reported, the elevated transaminases were mild and transient in most cases. The events reported were consistent with those seen in the Zolgensma clinical development program. One case of obstructing hydrocephalus was reported, see also under 'cases of special interest'. This was assessed as not suspected by investigator and sponsor. There were no clinically significant events of cardiac toxicity or sensory abnormalities related to DRG reported.

One patient experienced two adverse events of cardiac arrest: approximately 2 weeks after receiving Zolgensma, the patient developed multiple episodes of hypoxia which resulted in bradycardia requiring chest compressions for 1-2 minutes in duration. The patient experienced three of these events on consecutive days with changes in ventilator settings remediating the cardiac events. The same patient was reported to have a second event of cardiac arrest approximately 6 weeks after receiving Zolgensma: the patient had a bradycardic event with her bowel movement in the evening. The patient received chest compressions for about 1 minute. The patient responded well to bag ventilation with stabilisation of her vital signs. Both events were assessed as unrelated by the investigator and resolved with ventilatory adjustments.

Safety in special populations

To date, due to the low number of TEAEs considered related to Zolgensma, analysis of adverse events by intrinsic factors such as age, sex and race has not been performed.

No information is available regarding the safety of Zolgensma in patients with renal or hepatic impairment and in patients with pre-existing AAV9-antibodies above 1:50.

Immunological events (Study CL-101)

As expected, mean increases from baseline in anti-AAV9 titre were observed in all patients. In studies CL-303, CL-304 and CL-302, there was no development of antibodies to SMN protein following the administration of Zolgensma.

There is no apparent relationship between increases in anti AAV9 titre and increases in AST or ALT. Of the 4 patients that had significant increases in transaminases, titres elevations exceeding the level of quantification (> 1:819200) occurred at time points later than the reported adverse events of elevated transaminases. Similar elevations in anti AAV9 titre were also observed in patients that did not have

potentially clinically significant increases in transaminases. This suggests there is no relationship between post-baseline anti AAV9 antibody development and the potential for significant increases in transaminases.

T-cell Response to AAV9 and Human Survival Motor Neuron (hSMN)

Temporal relationships between AAV9 ELISpot values and AST and ALT elevations were qualitatively evaluated in the 4 patients with potentially clinically significant elevations of AST or ALT (> 3 × ULN). From this, ELISpot values did not reliably predict the potential for patients to have significant increases in transaminases. In the 11 patients that did not have potentially clinically significant increases in AST or ALT, ELISpot increases did not reliably predict potential for patients to have significant increases in transaminases.

Safety related to drug-drug interactions and other interactions

Based on the mechanism of action, occurrence of clinically-significant drug-drug interactions following Zolgensma administration is unlikely. Therefore drug-drug interactions have not been evaluated.

Experience with use of concomittant 5q SMA targeting agents such as nusinersen is limited.

Discontinuation due to AES

N/A

Post marketing experience

A cumulative search of safety data in the Novartis global safety database (Argus) through 31 December 2019 was conducted. This database contains all post-marketing safety and literature reports, including cases from ex-US Managed Access Program (MAP), patient-oriented programs (POP), as well as the French Temporary Authorisation for Use (ATU) procedure. As of 31 December 2019, a total of 192 cases with 488 AEs were retrieved from the Argus Safety database. Because of the limited information about the cases, e.g. concomitant medication, outcome, medical history etc., no firm conclusions that confirm or deviated from the known safety profile of AVXS-101 can be drawn. However, most reported events are consistent with the known adverse event profile of AVXS-101. In the list of spontaneous reports there seems a notable tendency of occurrence of pyrexia after administration of AVXS-101. The Applicant states that currently there is no explanation on that.

2.6.1. Discussion on clinical safety

From the safety database all the adverse reactions reported in clinical trials <and post-marketing> have been included in the Summary of Product Characteristics.

Adverse Reactions by MedDRA SOC/PT and Frequency	
Blood and lymphatic system disorders	
Common	Thrombocytopenia
Gastrointestinal disorders	
Common	Vomiting
General disorders and administration site conditions	
Common	Pyrexia

Adverse Reactions by MedDRA SOC/PT and Frequency	
Investigations	
Very Common	Transaminases increased
Common	Aspartate aminotransferase increased, alanine aminotransferase increased, troponin-I increased

The safety database of Zolgensma is small; at the cut-off date of 31 December 2019, 101 patients have received Zolgensma in clinical studies, via the proposed route of administration (IV) of which 98 patients received the proposed dose. Twelve of the 98 patients who received the proposed dose were treated with process A manufactured Zolgensma. Thus, 86 patients received AVXS-101 manufactured by process B via IV in the proposed therapeutic dose. In addition, 32 patients received Zolgensma via intrathecal route of administration at that data cut-off.

Most reported adverse events (AE's) were in the domains of Gastrointestinal disorders, General Disorders and Administration Site, Infections and Infestations, Injury, Poisoning and Procedural Complications, Respiratory, Thoracic and Mediastinal Disorders and Skin and Subcutaneous Tissue Disorders.

Some of the observed AE's may fit the almost natural occurring sign/symptoms in infants; diarrhoea, constipation, teething, gastroesophageal reflux disease, vomiting, otitis media and cough. Others may be disease related caused by weakness of intercostal muscles like respiratory events. Pyrexia was also quite common which may be unrelated to treatment. Eleven (14) patients (14.4%) were reported to have rash, of which none was considered serious. Onset of rash occurred around 6 months after treatment with Zolgensma and is therefore not considered to be related to treatment.

The most frequently occurring serious adverse event (N=14; 13.4%) across studies was pneumonia. Twelve patients (12.4%) had increased transaminases, for three patients (3.1%) this was considered as serious.

There is one case of development of communicating hydrocephalus in the clinical trial program, this case was assessed as possibly related by investigator and sponsor. In the post-marketing experience, there is one report of an obstructive hydrocephalus, this was assessed as not suspected by investigator and sponsor.

The single treatment arm study design makes it difficult to disentangle whether an adverse event is due to treatment with Zolgensma and accompanied corticosteroid use, due to SMA1 or its complications or due to natural occurring background childhood diseases.

AAV-therapy including AAV-9 vector are described in literature as a relatively safe treatment [11,12]. Liver, lung, skeletal muscle, CNS, heart and pancreas are tissues that have tropism for AAV-9[9]. For Zolgensma liver toxicity was observed. There were no overt indications pointing at lung, skeletal muscle, CNS, heart or pancreas involvement in the clinical programme. Biodistribution of both deceased patients shows that all analysed organs were targeted, with clear detection of Zolgensma vector genomes and RNA transcripts in all analysed organs. SMN protein expression detected in all regions of the spinal cord, at levels that were nearly equivalent and to non-SMA control spinal cord and in all regions of the brain. Motor neurons were similar in size and shape to the non-SMA controls.

[11] Saraiva, J., et al. (2016) Gene therapy for the CNS using AAVs: The impact of systemic delivery by AAV9. *Journal of Controlled Release*: 241: 94-109.
[12] Hocquemiller, M., et al (2016) Adeno-Associated Virus-based gene therapy for CNS diseases. *Human Gene Therapy*: 27: 478-496.

Because of the nonclinical dorsal root ganglia dell inflammation findings in a NHP study, the Applicant provided a critical assessment of collected data indicative for DRG impairment. For one deceased patient (study CL-303) a final histopathology report is provided. The histopathology report (addendum) of patient 303-001-001 showed no findings indicative of dorsal root ganglion (DRG) toxicity, in particular no considerable myelin loss and no spheroids in the white matter funiculi. The dorsal and ventral roots that were available for examination appeared unremarkable. For the other patient (i.e. from study CL-302), the Applicant provided a histopathology report and final immunostainings. This interim report concludes that the DRG (block B21) was abnormal for this patient: ganglion cell loss and excess small round cells. Also, there were some inflammatory cells. A cause could not be distinguished: 1) direct contribution of SMA, 2) secondary to hypoxic/ischaemic injury in the terminal illness of this patients, or 3) secondary to treatment with Zolgensma. The immunohistochemistry for block B21 has not determined the aetiology of the abnormalities in the DRG. The nonclinical finding of DRG inflammation reported in the non-human primate studies was not confirmed in clinical data thus far through the analysis of clinical data in either the Zolgensma IT or IV studies, as well as review of post-marketing safety data. Therefore, the clinical relevance of the DRG findings in non-human primate studies associated with IT administration of AAV vector gene therapies remains unknown. Nevertheless, sensory abnormalities suggestive of ganglionopathy is added to the list of events of special interest (AESIs). In addition, study protocols were amended to include additional age appropriate sensory testing, and call for attention to new symptoms of pain, numbness, or paraesthesia's as part of the neurologic exam at baseline and at each visit in all ongoing clinical trial protocols for Zolgensma.

The following AE's are of special interest:

Hepatobiliary abnormalities

One patient (E01-643) in study CL-101 received treatment without pre- or post-treatment with corticosteroids. The observed increase in transaminases led to a protocol adjustment including initiation of concomitant immunomodulatory regimen using prednisolone prior and continued after Zolgensma treatment for at least 30 days post-treatment in order to manage the host immune response to the AAV-derived therapy.

Increases in AST and ALT were observed in the majority of patients within two weeks after administration of Zolgensma irrespective of prednisolone pre- and posttreatment. Continuing elevations have been observed even under concomitant immunosuppression in two cases. However, in the majority these elevations recovered. For 14 patients increase in bilirubin was reported.

Haematological abnormalities

Non-serious and transient thrombocytopenia and lymphocyte count decreased were observed within 2 weeks after treatment with Zolgensma. Also, after IT administration abnormal haematology values were reported. Although not necessarily presumed, it is known that AAV9 might trigger systemic immune responses even after local administration. This would explain the abnormal haematological findings after IT administration in some degree.

Both hepatobiliary and haematology abnormalities that were observed after treatment with Zolgensma may be considered as an immune response to the viral capsid. To suppress the immune response a premedication prior to Zolgensma treatment and continued treatment with prednisolone is recommended. Thus far this treatment seems sufficient to suppress the immune response against the capsid.

Cardiac markers

In the majority of patients at baseline and during the study elevated CK-MB levels were observed. It seems that the course of the development of CK-MB values differs from patient to patient. In addition,

in study CL-101 Troponin-I was measured and half of patients had elevated levels after administration of Zolgensma. It should be noted that of these 8 patients, 2 (25.0%) had elevated cardiac Troponin I levels prior to administration of Zolgensma. In studies CL-304 and CL-302 Troponin-I is collected after protocol amendments. Data are available for 20 patients, of which 5 (25%) patients met the criteria for potentially clinically significant values.

From the data it seems that no pattern of CK-MB and Troponin-I in relation to Zolgensma treatment can be observed. The cardiac abnormalities that were found in the patients are in line with what is described in literature for SMA Type 1 patients. Cardiac development beyond 2 years of age for SMA Type 1 patients is unknown. However, there is a signal of cardiac toxicity that might be of clinical relevance mainly in view of the observed pre-clinical findings and cardiac comorbidities of initial disease.

Immunogenicity

An immune response to the adeno associated viral vector serotype 9 (AAV9) capsid occurred after infusion of Zolgensma despite the immunomodulatory regimen, including antibody formation against the AAV9 capsid and T-cell mediated immune response.

Patients with Anti-AAV9 titres ≥ 1:50 at baseline were excluded from the study. This cut-off was not based on a scientific rationale but rather on a professional recommendation. Ten patients had increases in anti-AAV9 titres to > 1:50 at week 1, with even greater increases thereafter. No patients returned to titres < 1:50 after dosing.

No immune response against the transgene protein was observed.

Mortality

Two deaths were reported per data cutoff. One patient died due to respiratory failure not related to Zolgensma and probably SMA1. The cause of death of the second patient was most likely due to hypoxic/ischemic brain damage due to respiratory tract infection requiring artificial ventilation. Respiratory insufficiency is a known feature of Spinal Muscular Atrophy Type 1.

Post-Marketing Experience

As of 31 December 2019, a total of 192 cases with 488 AEs were retrieved from the Argus Safety database. Because of the limited information about the cases, e.g. concomitant medication, outcome, medical history etc., no firm conclusions that confirm or deviated from the known safety profile of Zolgensma can be drawn. However, most reported events are consistent with the known adverse event profile of Zolgensma. In the list of spontaneous reports there seems a notable tendency of occurrence of pyrexia after administration of Zolgensma. The Applicant states that currently there is no explanation on that.

Long-term FU

The long-term follow up of the adverse events of AVXS-101 are unknown. The adverse events related to the immune response are not likely to have long term safety consequences. However, the long-term safety of gene expression is less clear. Though the carcinogenicity risk is considered low as AAV vectors mainly do not integrate in the host genome as compared to e.g. lentivirus, it can also not be fully excluded. Long-term data are needed to confirm this. Patients will be followed up till 15 years.

2.6.2. Conclusions on the clinical safety

Zolgensma is a relatively safe therapy with a mild and manageable safety profile. The safety profile of Zolgensma is mainly characterized by transient increases in transaminases and transient

thrombocytopenia. The safety profile is manageable with the prednisolone regime used in the clinical trials of 1 mg/kg/day.

The safety database is limited and there are uncertainties regarding a potential of cardiac toxicity and dorsal root ganglia (DRG) toxicity observed in non-clinical studies. Assessment of the cardiac safety is hampered by the observation that SMA Type 1 itself is associated with cardiac comorbidities. The nonclinical finding of DRG inflammation reported in the NHP studies is not confirmed in humans so far. Neither the autopsy reports of deceased study patients, nor evaluation of clinical data of IT or IV studies with Zolgensma, and review of the post-marketing safety data allow a conclusion on Zolgensma related DRG impairment.

On the long term, safety uncertainties with regards to gene therapy are undefined. This mainly pertains to the potential carcinogenicity.

The CAT considers the following measures necessary to address the missing safety data in the context of a conditional MA:

In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients younger than 6 months of age with Spinal Muscular Atrophy Type 1 with One or Two *SMN2* Copies the MAH should submit final data of study CL-303.

In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients younger than 6 months of age with Spinal Muscular Atrophy Type 1 with One or Two *SMN2* Copies the MAH should submit interim and final data of study CL-302.

In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in pre-symptomatic patients equal or younger than 6 weeks of age at time of treatment with SMA with bi-allelic deletion of *SMN1* with 2 or 3 copies of *SMN2*, the MAH should submit interim and final data of study CL-304.

In addition, the CAT considers the following measures necessary to address issues related to safety:

In order to further characterise and contextualise the outcomes of patients with a diagnosis of SMA, including long-term safety and efficacy of Zolgensma, the MAH should conduct and submit the results of a prospective observational registry AVXS-101-RG001 according to an agreed protocol.

The applicant should also perform a further evaluation of the finished product specifications when primary and key secondary endpoint data from additional patients with 2 copies of SMN2 are available (i.e. completion of CL-302 and CL-304 cohort 1). Based on this evaluation, it should be determined whether tightening of the release specification limits is needed to improve consistency of the batches and ensure optimal clinical outcome

The CHMP endorsed the CAT conclusion on clinical safety as described above.

Additional expert consultation

On September 6, 2019 a SAG Neurology meeting was convened upon request from the CAT. The following issues were discussed:

1. In the clinical study program, the applicant enrolled SMA type 1 patients which were defined by genotype (bi-allelic mutation of SMN1 and 2 SMN2 copies) and clinical symptoms (onset of clinical symptoms < 6 months of age). How well does this definition reflect the diagnostic approach in current clinical practice? In other words, how useful is this definition for the identification of the target population in clinical practice?

SAG experts considered that in real life setting, the application of these criteria may prevent potential patients with Type 1 SMA, for whom there is medical need, from receiving the treatment. The real-life situation will be that broader criteria will be used to define a patients as Type 1 SMA, as for example some 7-month old patients will also be diagnosed as Type 1 (as they will never learn to sit).

The patient representatives were concerned about the focus on the Type 1 definition as SMA is a spectrum disease and the division into types is artificial. Also, there were emerging data that the better definition of patient severity should be to talk about "non-sitters", "sitters" and "walkers". This position, however, was not shared by the present experts, first because it was not used for the pivotal studies, and also because it can't be predicted individually at an early stage. With regard to the proposal to extend the indication to include patients with 3 copies of SMN2, the experts were of the opinion that less than 20% of Type 1 patients will have such a copy number. They agreed that, based on the observed phenotype, usually SMN1 type c, these patients should be included.

2. The patients treated with Zolgensma clearly showed clinical benefit in terms of survival. How does the SAG value the motor milestone achievements so far of the treated patients in terms of clinical benefit?

Experts assigned a lot of clinical importance on the improvement expressed as motor milestone achievement, as it is considered that survival with no motor milestone achievement will not be considered as clinically valid treatment outcome in a number of EU countries, although cultural differences exist.

The Patient representatives reinforced the importance of respiratory effects, including the emergence of serious respiratory AE (pneumonia), improvements in swallowing and other relevant clinical signs, in the evaluation of the clinical benefit.

3. Having in mind that the current proposed indication for Zolgensma is "ZOLGENSMA is indicated for the treatment of 5q13 spinal muscular atrophy (SMA) Type 1":

a. The study population did not include patients older than 6 months of age. Is the SAG of the opinion that only SMA type 1 patients younger than 6 months of age would benefit from Zolgensma. would patients older than 6 months benefit? Is there an age above which patients would no longer benefit?

The SAG experts expressed a consensus that age should not be a factor restricting the treatment with Zolgensma. Data from other developments (Spinraza) show that patients over 6 mths still continue to experience benefits from treatment. Some raised the question on the permeability of the blood-brain barrier after the age of 6 mths, and how this may affect the effect size of the expected benefit from treatment, but since this is currently not known , there was a general agreement that there will be a number of eligible patients that will be older than 6 mths and will still benefit from treatment.

The preferable approach will be to be able to leave to the clinician in a dialogue with carers/family to determine the need for treatment, based on the motor, respiratory and general condition of the child.

b. What is the position of the SAG experts on the possibility to include in the indication the treatment of pre-symptomatic patients? Is this justified based on available data and the extrapolation of data from symptomatic patients? How will a SMA type 1 patient be defined based on genotype and do current clinical practices allow for the identification and management of these patients?

Experts were unanimous that treatment should start as early as possible, and pre-symptomatic patients, corresponding to the approved indication (in 5q SMA), should be treated immediately after genetic testing identification of biallelic mutations in SMN1.

4. How would the SAG experts define a patient in whom irreversible motor neuron loss has occurred to a point that no benefit can be reasonably expected on terms of motor development? How would such a patient population be identified?

In clinical practice, a combination of factors is used, including CHOP INTEND score, the presence of signs of choking when feeding, severe respiratory distress, severe muscular hypotonia and others, in order to determine when there is no benefit to be expected from any treatment. CMAP (Compound Muscle Action Potential) as a biomarker can be used as a guide (CMAP<1miliV is signalling severe neuro-muscular junction degeneration) but is so far not reliable on its own to serve as a decision tool for not starting treatment. The decision to treat or not is always to be discussed between the specialist and the patient's family.

The patient representatives stated that benefit is individual and anyone that can improve the course of their disease, moving it away from the expected natural course, should have the right to receive treatment.

The experts expressed concerns about patients with 1 SMN2 copy, who are unlikely to ever produce sufficient SMN protein to achieve meaningful milestones, resulting in a severely disabled patient with prolonged lifespan a very severe disease, and very low quality of life.

5. What is the SAG position on the consequences from the currently provided dosing recommendation restricted to patients in a weight range between 2.6 and 8.5 kg? Will this result in a restriction to the population that should be treated in your opinion?

SAG experts agreed that applying a weight range as a factor will result in an unnecessary restriction of the patient population and should not be done.

6. Does the SAG have any recommendations to study long term performance of Zolgensma, i.e. what DATA should be captured relating to efficacy and safety?

Longer than the proposed 18, 24 and 36 mths follow up was recommended (specific studies addressing this point need to be designed), including a combination of motor and intellectual function (performance and socialisation at school age- around 5-6 years, was mentioned as a time point for follow up), in order to gather data on the long term effects of Zolgensma. Additional data on indicators meaningful to patients and carers: fatigue, respiratory performance, swallowing, pain, functional cardiologic assessments (clinical indices are considered essential) CMAP evolution, days of hospitalisation, MRI of upper thigh regions, should be gathered.

It is important to state that the Type 1 patients should be with a much longer follow-up than proposed, as they will represent the greatest need to demonstrate a long term effect, and very little is known about the progression of treated Type 1 patients (particularly with 2 *SMN2* copies), as for those who have 3-4 *SMN2* copies, appears to be very different from the natural history.

PDCO FINAL ANSWERS:

1. What is the minimum duration needed for assessment of the effect of the drug in SMA types 1, 2, 3 and 4 (could be potentially administered from birth in pre-symptomatic patients).

2. In particular, what are the meaningful endpoints ("primary" and "secondary") to be captured which allow for long term assessment of efficacy for each of the SMA types? Are there prognostic factors?

The experts had difficulties answering these questions, as they represent a combination of different phenotypes, requiring separate evaluation of effect, which will occur in different time points. No consensus was presented with regard to the expected duration of studies, focusing on the different types of SMA.

2.7. Risk Management Plan

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Hepatotoxicity (Important Identified Risk)	**Routine risk minimisation measures**: SmPC section(s): 4.2, 4.4 4.8, 5.2 and 5.3 PL sections: 2, 3, 4 **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: Targeted follow up questionnaire **Additional pharmacovigilance activities**: AVXS-101-LT-001, AVXS-101-LT-002, AVXS-101-CL-303, AVXS-101-CL-304, AVXS-101-CL-302, and AVXS-101-RG-001
Transient thrombocytopenia (Important Identified Risk)	**Routine risk minimisation measures**: SmPC section(s): 4.4 and 4.8 PL sections: 2, 4 **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: Targeted follow up questionnaire **Additional pharmacovigilance activities**: AVXS-101-LT-001, AVXS-101-LT-002, AVXS-101-CL-303, AVXS-101-CL-304, AVXS-101-CL-302, and AVXS-101-RG-001
Cardiac adverse events (Important Potential Risk)	**Routine risk minimisation measures**: SmPC section(s): 4.4, 4.8, 5.2, 5.3 PL sections: 2, 4 **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: Targeted follow up questionnaire **Additional pharmacovigilance activities**: AVXS-101-LT-001, AVXS-101-LT-002, AVXS-101-CL-303, AVXS-101-CL-304, AVXS-101-CL-302, and AVXS-101-RG-001
Use in patients with anti-AAV9 antibody titres >1:50 and higher vector loads required (Important Potential Risk)	**Routine risk minimisation measures**: SmPC section(s): 4.2, 4.4, 4.8, 5.1 **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: None **Additional pharmacovigilance activities**: AVXS-101-RG-001
Dorsal root ganglia cell inflammation (Important Potential Risk)	**Routine risk minimisation measures**: SmPC section(s): 5.3 **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: Targeted follow-up questionnaire **Additional pharmacovigilance activities**: AVXS-101-LT-001, AVXS-101-LT-002, AVXS-101-CL-303, AVXS-101-CL-304, AVXS-101-CL-302, and AVXS-101-RG-001

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Long-term efficacy of onasemnogene abeparvovec therapy (Missing Information)	**Routine risk minimisation measures**: None **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: None **Additional pharmacovigilance activities**: AVXS-101-LT-001, AVXS-101-LT-002, and AVXS-101-RG-001
Risks related to off-label use for patients with >3 SMN2 copies i.e., higher prevalence of anti-AAV9 antibodies and higher vector loads required (Missing information)	**Routine risk minimisation measures**: None **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: None **Additional pharmacovigilance activities**: AVXS-101-RG-001

Conclusion

The CHMP, CAT and PRAC considered that the risk management plan version 0.7 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 24 May 2019. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant declared that onasemnogene abeparvovec has not been previously authorised in a medicinal product in the European Union.

The CAT/CHMP, based on the available data, considers onasemnogene abeparvovec to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Labelling exemptions

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group only for vial label component. The group rejected the request for the rest of the labelling particulars, i.e. generic outer carton sleeve and variable outer carton label. The Group requested that a bilingual option should be explored by the applicant regarding the outer carton, to accommodate the German language. The Group also rejected the proposed packaging configuration (generic outer carton sleeve + variable outer carton label)

The labelling subject to translation exemption as per the QRD Group decision above will, however, be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zolgensma (onasemnogene abeparvovec) is included in the additional monitoring list as:

- It contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU;
- It is a biological product that is not covered by the previous category and authorised after 1 January 2011;
- It is approved under a conditional marketing authorisation [REG Art 14-a]

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit risk assessment

3.1. Therapeutic Context

3.1.1. Disease or condition

Zolgensma (Zolgensma) is indicated for the treatment of patients with 5q spinal muscular atrophy with a bi-allelic mutation in the *SMN1* gene and either a clinical diagnosis of type 1 SMA or up to 3 copies of the *SMN2* gene.

SMA is an autosomal recessive, early childhood neuromuscular disease with an incidence of approximately 1: 10,000 live births, of which approximately 45-60% of cases are SMA Type 1. SMA patients lack the *SMN1* gene which leads to progressive loss of motor neurons and causes muscle weakness and death due to respiratory failure. Disease severity is negatively correlated with the amount of *SMN2* copies, with the majority of patients with type 1 having 2 copies. Of the patients with 3 copies of *SMN2*, based on natural history approximately 15% is expected to develop type 1 (will never be able to sit independently), 55% is expected to develop type 2 (will never be able to walk) and approximately 30% is expected to develop type 3a.

SMA type 1 is a fatal disease, and without respiratory support and tube feeding the majority of patients do not reach 2 years of age. Treatment is therefore aimed at prolonging survival and improving motor function.

No information considering the motor and cognitive development beyond the 2 years of life is available for patients with SMA type 1. Also, no information on complications in later life are available in these patients.

3.1.2. Available therapies and unmet medical need

There are limited treatment options for patients with SMA and treatment is mainly symptomatic and supportive, like orthopaedic surgery, physiotherapy and nutritional and ventilatory support.

The only available therapy to date is nusinersen (Spinraza), an antisense oligonucleotide (ASO) drug designed to increase the production of the SMN protein by modulating the splicing of the SMN2 gene. Treatment requires an induction period and regular maintenance dosing (once every 4 months). Nusinersen has safety considerations which require clinical monitoring, including thrombocytopenia, renal toxicity, coagulation abnormalities, and haemorrhagic complications of lumbar puncture and CSF removal. The need for repeat lumbar punctures may be challenging as SMA patients are likely to develop scoliosis, contractures, and often require spinal rod surgery and/or spinal fusion surgeries for continued survival and function. A recent publication described that approximately 30% of patients receiving nusinersen who complete the induction phase died compared to approximately 60% who received placebo. Nusinersen is indicated for the treatment of all SMA types (1-4).

3.1.3. Main clinical studies

The main study to support this application is study CL-303.

However, evidence of efficacy from the clinical program includes in addition to study CL-303, data from studies CL-101 cohort 2, CL-302 and CL-304 cohort 1. Pooling these studies, the clinical study program includes 81 subjects with 2 *SMN2* copies treated with the proposed dose. Fifteen patients with 3 *SMN2* copies are treated in cohort 2 of study CL-304. No patients with 1 copy of *SMN2* have been treated in the clinical studies. One patient with 4 *SMN2* copies has been treated in study CL-304 cohort 3.

Study CL-303 is a Phase 3, open-label, single-arm, single-dose study of Zolgensma that enrolled 22 patients with SMA Type 1 who were either symptomatic or pre-symptomatic with no functional *SMN1* gene as well as 1 or 2 copies of *SMN2* and who are < 6 months (< 180 days) of age at the time of gene replacement therapy (Day 1). Co-primary endpoints were:

- The proportion of patients that achieve functional independent sitting for at least 30 seconds at the 18 months of age study visit.
- Survival at 14 months of age. Survival is defined by the avoidance of the combined endpoint of either (a) death or (b) permanent ventilation, which is defined by tracheostomy or by the requirement of ≥ 16 hours of respiratory assistance per day (via non-invasive ventilatory support) for ≥ 14 consecutive days in the absence of an acute reversible illness, excluding perioperative ventilation. Permanent ventilation, so defined, is considered a surrogate for death.

Since SMA patients have very limited motor skills, it is challenging to assess motor ability in these patients. The CHOP-INTEND (The Children's Hospital of Philadelphia Infant Test for Neuromuscular Disorders) score is a sensitive score for motor function ability below formal motor milestones designed and validated specifically for SMA patients with a maximum score of 64. The CHOP-INTEND score

represents measures of disease severity. The CHOP-INTEND score was included as a secondary and exploratory endpoint in the Zolgensma clinical studies.

Study CL-101, was an open-label, dose-escalating study investigating the safety and efficacy of a single intravenous infusion of AVSX-101 in 15 patients diagnosed with SMA type 1. Three patients received a low dose of 6.7E13 vg/kg (cohort 1) and 12 patients received an intermediate dose of 2.0E14 vg/kg (cohort 2, equivalent the proposed therapeutic dose, 1.1E14 vg/kg). Survival without permanent ventilation at 13.6 months of age was the primary endpoint in study CL-101.

Study LT-001 is an ongoing, observational, long-term, safety follow-up study of patients who completed Study CL-101. Of the 15 patients from study CL-101, 13 patients were enrolled in study LT-001 (3 from cohort 1 and 10 from cohort 2). The study consists of an initial 5-year phase, during which patients are being seen annually for evaluation of long-term safety, followed by a 10-year observational phase.

Study CL-302 is the European equivalent of study CL-303 with a similar design. Thirty-two patients with SMA Type 1 who were either symptomatic or pre-symptomatic with no functional *SMN1* gene as well as 1 or 2 copies of *SMN2* and who are < 6 months (< 180 days) of age at the time of gene replacement therapy (Day 1) were enrolled and treated. One patient meeting all other criteria was treated at 181 days of age and is currently not reflected in the ITT population. The study is still ongoing and only preliminary data is available.

Study CL-304 is a study including pre-symptomatic patients with 2 (n=14, cohort 1) or 3 (n=15, cohort 2) *SMN2* copies. Currently, information from this study is limited also. Data from this study is submitted to support the treatment of pre-symptomatic patients with 2 or 3 *SMN2* copies.

3.2. Favourable effects

Study CL-303

Of the 22 patients enrolled in study CL-303, 20/22 patients were reported alive and without permanent ventilation at 13.6 months of age. One patient was deceased, one patient withdrew due an AE but was reported to be event free at the time of withdrawal and one patient was reported to be on permanent ventilation prior to withdrawal.

Patients treated with Zolgensma achieved motor milestones which are never achieved in natural history. Thirteen out of 22 patients had reached the milestone of independent sitting at 18 months of age. One patient had reached the milestone of walking with assistance.

Formal evaluation of the co-primary endpoints, i.e. event-free survival at 14 months of age and independent sitting at the 18 months of age visit is outstanding.

All patients showed increase in CHOP-INTEND score from baseline during the study. The mean (SD) increases (improvement) from baseline to Month 1, Month 3, Month 6 and Month 12 after the administration of Zolgensma were 6.9 (5.35), 11.7 (6.40), 14.6 (7.04) and 16.4 (8.04) points, respectively. CHOP-INTEND scores at 18 months of age (end of study visit) were available for 16 patients. The mean total score (SD) was 51.2 (5.67) and the mean change from baseline (SD) was 19.3 (9.13). Twenty-one patients (95.5%) achieved a CHOP-INTEND score of ≥40, 14 (63,6%) achieved a score of ≥50 and 5 patients (22,7%) achieved a score ≥60 at any point of time in the study.

At baseline, all patients were able to swallow thin liquids, and none required feeding or ventilator support. Eighteen of 22 patients (81.8%) were independent of ventilatory support (as assessed by Trilogy BiPAP data) at 18 months of age (co-secondary endpoint, p< 0.0001). In total, 15 of 22 patients (68.1%) did not require any non-invasive ventilatory support at any point during the study.

Fifteen (15) of 22 patients (68.1%) received no non-oral feeding support at any time during the study. Seven patients (31.8%) received feeding support during the study of which 2 patients needing a more sustained support (Nissen fundoplication) A total of 19 of 22 patients (86.3%) were feeding without mechanical support at the end of the study (or early termination).

The ability to thrive at 18 months of age was defined as the ability to tolerate thin liquids, does not receive nutrition through mechanical support, and maintains weight consistent with age. Nine of 22 patients (40.9%) met the ability to thrive criteria at 18 months of age.

Based on the literature available it can be concluded that few if any patients suffering from SMA type 1 survive the first 18 months of life without any ventilatory requirement or nutritional support.

The data from study CL-303 is supported by study CL-101, and the preliminary data of study CL-302 and CL-304 cohort 1.

Study CL-101

Of the 12 patients treated with the therapeutic dose (cohort 2.2.0E14 vg/kg) of Zolgensma in study CL-101, 100% was alive without permanent ventilation at 13.6 months of age. At 20 months of age and at 24 months post-dose, this effect was sustained since 100% of the patients was alive without permanent ventilation versus 8% in the natural history control.

At Months 1 and 3, patients in Cohort 2 had a mean *improvement in CHOP-INTEND score* from baseline of 9.8 and 15.4 points, respectively (n=12, both $p<0.001$). At Month 12, the mean improvement IN CHOP-INTEND score was 25.7 points in cohort 2 patients that were treated <6 months of age (n=9, $p<0.001$).

Nine patients gained the ability to sit without support for ≥ 30 seconds. Two patients gained the ability to walk alone. In the long term follow up study, the achieved motor milestones were maintained for the majority of patients.

Ability to thrive and non-invasive ventilatory support stabilized or improved in the majority of the patients in cohort 2.

Study CL-302

At the time of the 31 DEC 2019 data cut, of the 33 enrolled patients, 32 patients (93.6%) had had survived without permanent ventilation. Eighteen patients were ≥ 14 months of age and 4 were ≥ 18 months of age.

Eight out of 33 patients reached the milestone of sitting independently for 30 seconds.

At month 6, the mean improvement in CHOP-INTEND score from baseline was 13.3 (6.54) points.

Study 304

At the time of their most recent visit prior to data cutoff, patients in the 2-copy cohort had been in the study for an average 10.5 months (range: 5.1 to 18 months)For the 14 non-symptomatic subjects with 2 SMN2 copies treated with Zolgensma a normal motor development (i.e. Bayley score and motor milestone achievement) is observed up to 18.6 months after treatment. All patients are alive without permanent ventilation and none requires feeding or ventilatory support.

Patients in the 3-copy cohort had been in the study for an average 8.74 months (range: 2 to 13.9 months). During the follow up time the motor development is within the normal limits and all patients are alive without permanent ventilation.

3.3. Uncertainties and limitations about favourable effects

No patients with 1 *SMN2* copy were enrolled in the clinical studies.

For patients with 3 *SMN2* copies, only pre-symptomatic patients were enrolled and follow up is limited (up to 14 months).

There are important uncertainties about the long-term efficacy of Zolgensma, commercial finished product. The database currently contains a limited follow-up for the 101 patients treated with an IV dose. In the follow up of CL-303 from the previous data cut until the end of the study only a limited amount of new milestones were recorded and only one patient achieved the milestone of walking alone.

Information regarding benefit in these patient populations will be gathered in study CL-304 and the registry study AVXS-101-RG-001 as part of the conditions.

In general, the historical controls with 2 *SMN2* copies are considered adequate for comparison with the study population. Although there are indications that the patients in the PNCR cohort have less severe disease (as expressed by the older age of the included patients), this is not considered a major issue since the potential bias this creates i.e. these patients are expected to experience the event earlier, is not in favour of Zolgensma. Based on the literature on this topic it can be concluded that few if any patients suffering from SMA type 1 survive the first 18 months of life without any ventilatory requirement and/or nutritional support. Also based on literature it can be concluded that few (effectively zero) patients suffering from SMA type 1 would be expected to be able to effectively swallow safely and maintain weight within normal growth parameters absent the provision of mechanical nutrition support through 18 months of age.

The characterisation of the natural history for patients with 3 *SMN2* copies is limited. The natural history of this patient group is very heterogeneous. Within the population of patients with 3 *SMN2* copies about 15% patients present with the clinical picture of a type 1 patient, about 55% presents with type 2 and the remaining patients (about 30%) present with the clinical picture of type 3a (Calucho et al 2018). Not only the observed heterogeneity but also the (relative) short follow-up (on average 8.74 months) makes it difficult to draw conclusions on the efficacy of the pre-symptomatic patients included in study CL-304. The applicant has committed to further characterize the natural history for patients with 3 *SMN2* copies (especially for the patients presenting with a clinical picture of type 2 and 3a SMA).

No information is available on the cognitive development of the SMA patients. The applicant has committed to aim to record these parameters in the registry study. In study CL-303, cognitive subset and expressive communication subset assessment of the Bayley scales were performed. However, no analysis was done for these variables. This analysis will be presented as an exploratory analysis in the final CSR of study CL-303.

There is uncertainty about the effective dose that was received by the patients, as the infectious titre calculated per 10^{13} vector genomes (vg) shows relatively large batch-to-batch variation and vector genome titre decreases during storage of the product. The decrease in genomic titre during storage also hampers potency control as potency is expressed relative to a stored reference batch. This may have affected consistency of the potency of Process B batches, although the clinical impact of the potential differences in potency is unknown. As differences in effective dose could (partly) explain the variation in the CHOP-INTEND seen in the clinical studies (CL-302, -303, and -304) the applicant committed to further analyse whether tightening of the acceptance criteria for quality parameters is needed to ensure optimal clinical outcome.

3.4. Unfavourable effects

The safety profile of Zolgensma was studied in 101 patients. In 98 patients the proposed dose via the proposed route of administration (IV) was administrated. Twelve of the 98 patients who received the proposed dose were treated with process A manufactured Zolgensma. Thus, 86 patients received AVXS-10 manufactured by process B via IV in the proposed therapeutic dose. Of these 98 patients, 97 (99%) were reported to experience any adverse event (AE). Most reported AE's were in the domains of Gastrointestinal disorders, General Disorders and Administration Site, Infections and Infestations, Injury, Poisoning and Procedural Complications, Respiratory, Thoracic and Mediastinal Disorders, and Skin and Subcutaneous Tissue Disorders.

The most frequently occurring serious adverse event (SAE) across studies was pneumonia (N=13). Based on pre-clinical data, the Applicant pre-defined elevated liver enzymes, cardiac markers and dorsal root ganglia cell inflammation as AE's of special interest to be followed up.

Increased transaminases (AST and ALT) were observed in the vast majority of patients within 2 weeks after administration of Zolgensma. In most cases these elevations were transient. Twelve patients (12.4%) had increased transaminases, for three patients (3.1%) this was considered serious. A case of serious liver failure in a USA-patient treated with Zolgensma in addition to Spinraza (nusinersen) was described.

Both haematology and hepatobiliary abnormalities that were observed within two weeks after treatment with Zolgensma may be considered as an immune response to the viral capsid. Increase in transaminases observed in the first treated patient led to a protocol amendment of standard immunomodulatory regimen using prednisolone prior to and after Zolgensma administration, which seems sufficient to reduce the immune response.

In the majority of patients at baseline and during the study elevated CK-MB levels were observed. All observations were considered clinically asymptomatic. In addition, in study CL-101 Troponin-1 was measured and half of patients had elevated levels after administration of Zolgensma. After a protocol amendment Troponin-I is measured in studies CL-304 and CL-302. From the CK-MB and (limited) Troponin-I data no clear pattern can be distinguished.

Non-serious and transient thrombocytopenia (N=4, for 1 patient this was considered a serious AE) and lymphocyte count decreased (N=2) were observed within 2 weeks after treatment with Zolgensma. For 14 patients increase in bilirubin was reported.

In two patient's abnormal haematology values were reported after more than one year: eosinophil count increased that resolved and for one patient haemoglobin decrease which resulted in an ongoing anaemia. In long-term study LT-001 some patients were reported to have abnormal post-baseline haematology values.

One patient in study CL-303 and one patient in study CL-302 deceased during the study period. In both cases the cause of death was likely due to complications of the disease.

All patients in clinical trials developed an immune response against the AAV9 capsid. No immune response against the transgene protein was observed.

Post-marketing experience is consistent with the known adverse event profile of Zolgensma.

It is expected that information regarding these events will be followed in the registry study AVXS-101-RG-001.

3.5. Uncertainties and limitations about unfavourable effects

The clinical trial safety database for Zolgensma is small: data for 98 patients was submitted (data cut-off 31 December 2019), to which Zolgensma was administrated in the proposed dose via the proposed route of administration (IV). Of those 98 patients, 86 patients received Zolgensma that was manufactured by process B.

Based on two biodistribution reports it is concluded that that Zolgensma was distributed to the brain. Because of the dorsal root ganglia dell inflammation findings in an NHP study, the Applicant provided a critical assessment of collected data indicative for DRG impairment. For both deceased patients (i.e. from study CL-303) a histopathology report is provided. This report showed no findings indicative of dorsal root ganglion (DRG) toxicity. For patient (from study CL-302) a histopathology report and final immunostainings were provided. This report showed findings indicative of DRG toxicity. A cause for this finding could not be distinguished: 1) direct contribution of SMA, 2) secondary to hypoxic/ischaemic injury in the terminal illness of this patients, or 3) secondary to treatment with Zolgensma. The additional immunohistochemistry has not determined the aetiology of the abnormalities in the DRG and the appearances of the few inflammatory cells would probably argue against a significant ongoing inflammatory process. The nonclinical finding of DRG inflammation reported in the non-human primate studies was not confirmed by clinical data thus far. Therefore, the clinical relevance of the DRG findings in non-human primate studies associated with IT administration of AAV vector gene therapies remains unknown. Nevertheless, sensory abnormalities suggestive of ganglionopathy is added to the list of events of special interest (AESIs). In addition, study protocols were amended to include additional age appropriate sensory testing, and call for attention to new symptoms of pain, numbness, or paraesthesia's as part of the neurologic exam at baseline and at each visit in all ongoing clinical trial protocols for Zolgensma.

There may be a potential for cardiovascular toxicity of Zolgensma based on non-clinical studies and the observed increase of troponin-I and CK-MB in clinical studies. Given the high rate of inborn cardiac failures as concomitant disease at children with SMA it is unclear whether the cardiovascular effects are due to the disease or the administration of Zolgensma.

The single treatment arm clinical study design makes it difficult to disentangle whether an adverse event is due to treatment with Zolgensma, due and accompanied corticosteroid use, due to SMA type 1 or its complications or due to naturally occurring background childhood diseases. The increased transaminases is an exception as this is in general reported within a short time after Zolgensma treatment.

One patient experienced a SAE of communicating hydrocephalus in the clinical trial program. Another case of an obstructive hydrocephalus was reported post marketing. Based on the current evidence it is endorsed that any new cases of hydrocephalus should be subject to thorough follow-up. In the context of routine pharmacovigilance, the applicant is expected to discuss any unexpected findings or trends in the PSURs.

Concerning AAV vector therapy uncertainties of the safety profile, in general, exist in the long term. The risk of carcinogenicity might be theoretical, but it is currently unknown and cannot be ruled out as the life expectancy of the patient increases. As described in the current EMA guideline on Advanced Therapy Medicinal Products (EMEA/1499965/2008) risks related to persistence of the product in patients should include malignancies as late implication.

3.6. Effects Table

Table 28: Effects Table for Zolgensma (data cut-off: 31 DEC 2019)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects: 2 SMN2 copies						
Co-primary endpoint: Survival at 13.6 months of age	Not dead or with permanent ventilation	n/N (%) of patients	20/22 (90.0%)	25%	SoE: supported by data from study CL-101. SoE matching to natural history cohort. Un: Batch to batch consistency within process B is uncertain; some contribution to the heterogeneity of response cannot be excluded	Study CL-303
3.7. Co-primary endpoint: proportion of patients achieving independent sitting	Sits alone ≥ 30 sec	n/N	14/22 (63.6%)	none	SoE: milestones never reached in natural history. Supported by data from study CL-101, CL-302 and CL-304. One of 22 patients is walking alone. Un: continuation of development uncertain. Un: Batch to batch consistency within process B is uncertain; some contribution to the heterogeneity of response cannot be excluded	Study CL-303
CHOP-INTEND at 18 months end of study visit	Change from baseline Total score	Score Mean (SD) Score Mean (SD)	19.3 (9.13) 51.2 (5.67)	-	SoE: sensitive scale, designed for SMA. Supported by data from study CL-101, CL-302 and CL-304 Un: only available for 16 patients Batch to batch consistency within process B is uncertain; some contribution to the heterogeneity of response cannot be excluded	Study CL-303
Favourable effects: 3 SMN2 copies						
Highest Motor milestone achievement	Stands up Walks with assistance Walks alone	n/N	9/15 7/15 3/15	unknown	SoE: consistent with data from 2 SMN2 copies Un: does not exceed natural history due to heterogeneity in phenotype, Follow-up is to short.	CL-304,cohort 2
Unfavourable Effects*						
Elevated AST >3x ULN		%	14.3	N/A	SoE: Observed within 2 weeks after treatment	Summary of Clinical Safety
Elevated ALT >3x ULN		%	15.3	N/A	SoE: Observed within 2 weeks after treatment	Summary of Clinical Safety
Total bilirubin >1.5x ULN		%	14.3	N/A	Un: grade 3 bilirubin was reported before treatment with Zolgensma and seemed to decrease over time	Summary of Clinical Safety
Very low platelets		%	7.1	N/A	SoE: Observed within 2 weeks after treatment	Summary of Clinical Safety
Elevated CK-MB**		%	88.2	N/A	Un: at baseline most patients already had elevated CK-MB levels	Summary of Clinical Safety

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Troponin-I >0.05***		%	15.6	N/A	Un: 25% of patients in study CL-101 had elevated troponin-I levels at baseline. For study CL-304 and CL-302 no pre-dosing troponin-I data is available for the majority of patients	Summary of Clinical Safety
Antibodies against AAV9		%	100	N/A	Un: limiting effect on efficacy	RPT-773

Abbreviations: AST = Aspartate Aminotransferase, ALT= alanine aminotransferase, CK-MB = CK isoenzyme-MB
Notes:
*Safety database consists of 98 patients who received the proposed therapeutic dose via the proposed route of administration.
** Data available for 85 patients.
*** Only investigated in Study CL-101 (N=12) and after protocol amendment in newly enrolled patients in studies CL-304 and CL-302 (N=20).

3.8. Benefit-risk assessment and discussion

3.8.1. Importance of favourable and unfavourable effects

Importance of favourable effects and the associated uncertainties

As untreated severe 5q SMA 1 is a lethal disease and the majority of patients die before the age of 2, the primary endpoint of survival without permanent ventilation is considered clinically meaningful for patients with 2 *SMN2* copies. The difference in survival during and at the end of the study between the patients treated with Zolgensma and natural history in the patient population with 2 copies of *SMN2* is evident in all submitted clinical studies in which patients have a long enough follow-up.

Motor milestone achievement was monitored in the studies. In the natural history of patients with 2 *SMN2* copies, the milestone of sitting independently is never reached. The achievement of this motor milestone and all subsequent milestones is therefore clinically relevant. There is however a significant proportion of patients that have not reached motor milestones, or only limited ones (solely head control within 18 months follow up). This information is crucial for caregivers and physicians to make an informed decision and is adequately represented in the SmPC.

The natural history for patients with 3 *SMN2* copies is very heterogeneous. Of the pre-symptomatic patients, 15% are expected to develop SMA type 1 and never reach the motor milestone of independent sitting. Fifty-five % are expected to never stand up and learn to walk (SMA type 2) and 30% will learn to walk but lose this motor milestone (SMA type 3a). Motor milestone achievement in patients with 3 copies of *SMN2* is therefore difficult to interpret especially given the short follow up.

The majority of the patients with 2 *SMN2* copies had significant increases in the CHOP-INTEND score. CHOP-INTEND scores ≥40 are never reached in untreated SMA patients above 6 months of age. Since this is a sensitive scale almost exclusively designed for and validated in SMA patients, this is considered clinically meaningful. However, after 9 months of treatment the CHOP-INTEND stabilises around a score of 50 and the score is not predictive for motor milestone achievement. The consequences for the further motor development of the child are currently unknown and this should be followed up. The expected motor development should be discussed as this is important for an informed decision. The few asymptomatic patients (N=10) with 2 *SMN2* copies follow a near normal motor development reaching the maximal score in about 9 months after treatment. However, CHOP-INTEND scores in pre-symptomatic patients with 2 or 3 SMN2 copies is of less relevance since these patients exceed the maximum motor function which can be measured with the CHOP-INTEND.

In study CL-303, Bayley scale assessment was conducted from baseline. This motor function scale is clinically relevant since it is norm-referenced and does thus provide insight in the development of treated patients compared to healthy peers. Comparing the gross motor scaled scores of symptomatic, diseased patients with SMA type 1 to those of healthy, unaffected peers indicates that SMA patients score poorly despite receiving some benefit from gene replacement therapy. This information is considered important and is included in the SmPC. Gross and fine motor development in pre-symptomatic patients with 2 copies of *SMN2* is largely developmentally appropriate and similar to neurologically normal peers.

The effects on ventilatory and nutritional support are important and clinically relevant since this has a large impact on daily life and quality of life for patients. The submitted data indicates that patients with 2 *SMN2* copies treated with Zolgensma need significantly less ventilatory and nutritional support than can be expected based on natural history. Considering the pre-symptomatic patients only limited data are available. The information available suggest a normal motor development pattern up to 6 months after treatment for patients with 2 *SMN2* copies. However, the number of patients as well the time of follow-up does not allow for robust conclusions to be drawn. This also holds for the pre-symptomatic patients with 3 *SMN2* copies. The natural history for these patients is very heterogeneous. In patients with type 2 or 3a it would be more informative to monitor deterioration and loss of milestones however this is not possible given the short follow up time of the study.

Nevertheless, there is strong medical need as well in patients with 3 *SMN2* copies given that 15% of these patients is expected to develop a SMA type 1 phenotype. The large increase in benefit associated with earlier (pre-symptomatic) treatment makes it unfeasible to wait until symptoms arise to decide which patients to treat. In addition, also (pre)-symptomatic patients with SMA type 2 are expected to benefit from treatment. Therefore, benefit can be extrapolated to patients with 3 *SMN2* copies.Confirmation of the benefit is expected to come from study CL-304 as part of the specific obligations.

The uncertainty about long term efficacy is of importance. Data on maintenance of efficacy of the process B product will have to come from the planned long term follow up studies that are part of the RMP. It is of importance to adequately assess motor-neurodevelopment, cognition, language development and quality of life in treated patients in the long-term follow-up studies. This is especially important given the uncertainties surrounding the comorbidities that might arise in aging patients since normally SMA type 1 patients do not live past 2 years of age. Variables of interest for caregiver and physician are the expectations later in life such as but not limited to; which are the milestones that might be reached, subgroups of patients with better or worse results after treatment, predictive characteristics and so on. This information is critical for an informed decision, should be analysed and included in the SmPC in due time.

In the long-term follow-up study LT-001, seven out of 13 patients started treatment with nusinersen (Spinraza). For these cases it is difficult to disentangle whether maintenance of effect or further improvement is due to Zolgensma, nusinersen or an additive effect. Further analysis of these patients suggests that the improvement with Zolgensma did not meet the expectations of caregivers and treating physicians and additional treatment with nusinersen was started. This did not add to the improvement in most patients.

During the clinical development program of Zolgensma, the manufacturing process was changed. Product manufactured with process A was used in study CL-101 while the product manufactured by process B was used for the subsequent studies. Uncertainties regarding the quality of process A cannot be retrospectively solved and comparability between the two production processes cannot be concluded. In addition, there are uncertainties with regard to the effective dose that was received by the patients, as vector genome titre decreases during storage of the product. This may have affected consistency of the Process B batches and raises questions regarding the ability to supply patients with a finished product

that is within the therapeutic range (see quality part). As differences in the effective dose could (partly) explain the variation seen in the clinical studies (CL-302, CL-303, and CL-304) the applicant committed to further analyse whether tightening of the acceptance criteria for quality parameters is needed to ensure optimal clinical outcome.

Importance of unfavourable effects and the associated uncertainties

The safety database is for Zolgensma is small, however a consistent safety profile is observed.

Treatment with Zolgensma is associated with increased liver transaminases indicative of a cytotoxic T-cell response directed against transduced liver cells. It is important to adequately manage the observed liver transaminase increase to prevent hepatotoxicity.

The potential for cardiotoxicity is of importance since cardiovascular effects were observed in pre-clinical studies, accompanied by elevations in cardiac markers in the clinical studies. In this perspective, Troponin-I data is important as it is considered a sensitive marker of cardiac injury. Another pre-clinical finding is dorsal root ganglia toxicity. Currently, this is not confirmed by clinical data. Sensory abnormalities suggestive of ganglionopathy is added to the list of events of special interest (AESIs) and part of the neurological examination. The potential cardiotoxicity and DRG toxicity, although both important, needs to be viewed in the light of the live-saving character of this treatment.

Given the general uncertainties about the potential risks of gene therapy, long term monitoring of safety is necessary. This mainly pertains to the monitoring of carcinogenicity, which at the moment, is considered a low risk since AAV vectors do not integrate in the host genome.

Currently, it is uncertain whether there is an influence of batch to batch differences of process B manufactured Zolgensma on the safety profile.

The long-term safety and efficacy of Zolgensma will be followed up in the prospective observational registry AVXS-101-RG-001.

3.8.2. Balance of benefits and risks

The efficacy of the Zolgensma process B product for the treatment of patients with a bi-allelic mutation in SMN1 and 2 copies of *SMN2* (SMA type 1 phenotype) is based on improvement in survival that exceeds the expectations given the natural history of the disease. Symptomatic patients with 2 *SMN2* copies are expected to benefit from treatment with regard to achievement of motor milestones, i.e., will reach motor milestones otherwise observed with a less severe SMA phenotype. Preliminary data suggest that the motor development is plateauing after the sitting milestone is reached. The patients that benefit most from Zolgensma treatment, i.e. the pre-symptomatic patients, showed improvement in motor-development approaching that of healthy peers. As clinical experts assigned a lot of importance on the improvement expressed as motor milestone achievement, and as it is considered that survival with no motor milestone achievement limits the clinically validity of the treatment outcome (although cultural differences exist), it is considered to follow the motor development in these patients. Based on the current available information and respecting the cultural differences an indication including clinically diagnosed type 1 SMA patients and those subjects with 2 *SMN2* copies is considered acceptable.

Given the limited follow-up in patients with 3 *SMN2* copies, benefit cannot be substantiated by data. However, the combined 5q pathomechanism/Zolgensma mode of action results in potential higher benefit in the presence of quantitatively more viable motor neurons. Patients with 3 copies of *SMN2* can reasonably be expected to have a higher number of viable motor neurons compared to an age matched patient with 2 copies of *SMN2*. Extrapolation from the *2 SMN2* copy data to *3 SMN2* copy patients seems possible and justifiable given the expected progression of the disease (15% will develop type 1 and 50% will develop type 2 SMA). Further, it is assumed that the results obtained with the populations studied

can be extrapolated to patients with type 2 SMA. Withholding treatment from these patients would leave a considerable number of type 1 patients and type 2 patients in need of treatment untreated. Treatment of these patients with 3 SMN2 copies is not outweighed by the treatment of patients with 3 SMN2 copies that might be expected to develop a milder form of SMA (type 3a, approximately 30%) for which the benefit –risk of the treatment with Zolgensma remains to be established.

No patients were enrolled and treated in the studies with 1 copy of *SMN2*. Although there are concerns that treatment in the majority of patients is futile, the warnings in the SmPC are considered sufficient to guide an informed decision on a case-by-case basis on which patient might benefit from treatment.

There is a concern that an indication based on the genetic diagnosis will exclude patients presenting with a severe phenotype despite having more than 3 copies of the *SMN2* gene. It is therefore considered necessary to include the clinical diagnosis of SMA type 1 as follows:

Zolgensma is indicated for the treatment of:

- patients with 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene and a clinical diagnosis of SMA type 1, or

- patients with 5q SMA with a bi-allelic mutation in the SMN1 gene and up to 3 copies of the SMN2 gene.

Zolgensma was shown to be relatively safe. The adverse effects following dosing with Zolgensma could be primarily contributed to the immune response against the AAV9 capsid. These events, elevated liver transaminases and thrombocytopenia were sufficiently managed with a prednisolone regimen used in the clinical trials.

Concerns about possible cardiovascular and DRG toxicity have been solved after the provision of a cardiac safety report, a clinical impact assessment of DRG toxicity, immunohistochemistry reports of the two deceased patients and measures to monitor treated patients for symptoms indicative for DRG adverse events.

From a quality perspective, uncertainties with regard to control of the effective dose remain, as genomic titre decreases during storage and no stable reference standard is available to control the potency of the product. However, consistency of the potency measurement will be further ensured for commercial manufacturing by the implementation of an assay control and adequate monitoring of stability and performance of the reference standard. In addition, the applicant committed to perform next generation sequencing on the first 30 commercial batches, which will provide assurance of vector genome integrity and, together with proof of vector infectivity and therapeutic protein expression, indirectly, biological activity. The applicant's evaluation of batch release data and the relation between clinical outcome and FP attributes did not identify a need to tighten the FP specifications. However, clinical data are considered currently too limited to reach a final conclusion. The applicant committed to perform a further evaluation of the FP specifications when additional patient data are available. Based on this evaluation it should be determined whether tightening of the release specifications limits is needed to improve consistency of the batches and ensure optimal clinical outcome.

In the current medical treatment arsenal for patients with SMA type 1 there is still an unmet medical need.

3.8.3. Additional considerations

A Scientific Advisory Group was convened at the request of the Committees.

The SAG experts expressed a consensus that age and weight should not be a factor restricting the treatment. Experts were unanimous that treatment should start as early as possible, and pre-

symptomatic patients, corresponding to the approved indication (in 5q SMA), should be treated immediately after genetic testing identification of bi-allelic mutations in *SMN1*.

In clinical practice, a combination of characteristics is used, including CHOP INTEND score, the presence of signs of choking when feeding, severe respiratory distress, severe muscular hypotonia and others, in order to determine when there is no benefit to be expected from any treatment.

The dose finding for Zolgensma was not optimal, leading to uncertainties about the optimal effective and tolerable dose. In addition, the Applicant is conducting clinical studies investigating the intrathecal administration of Zolgensma in the treatment of SMA type 2. Intrathecal infusion might be effective in lower total dosages and might also lead to a less severe immune response. However, currently these studies are on hold as unexpected serious adverse events are observes in cynomolgus monkeys (inflammation of the spinal ganglions).

The only other approved therapy for SMA at this moment is nusinersen (Spinraza). Efficacy of nusinersen was based on several clinical studies, among one phase 3 study in which nusinersen was compared to placebo in a multicentre, randomized, double-blind, sham-procedure controlled study in 121 patients with bi-allelic deletion of *SMN1*, 2 *SMN2* copies and age of onset ≤6 months of age.

27/80 (34%) patients who died or required permanent ventilation on nusinersen compared to 20/41 (49%) on control. There were 12/80 (15%) deaths on nusinersen, compared to 13 (32%) on control. A statistically significantly higher percentage of subjects achieved a CHOP INTEND response in the nusinersen group (71%) compared to the control group (3%; $p<0.0001$). Several of the infants treated with nusinersen had improvements of 10 points or more on the CHOP INTEND total score. Sixteen (22%) achieved full head control, six (8%) achieved independent sitting and one achieved standing with support. Except for one subject, all subjects in the control group showed no change or worsened. Although it is clear patients benefit from nusinersen treatment, indirect comparison with the results of nusinersen indicate that the effects on survival, CHOP-INTEND score and motor milestone achievement of Zolgensma supersede the effects of nusinersen in the SMA type 1 population. In addition, the single treatment of Zolgensma is an advantage for the patients compared to the need for repeated treatment with nusinersen.

Conditional marketing authorisation

During the evaluation, the applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14-a of the above-mentioned Regulation. The product falls within the scope of Regulation (EC) No 507/2006 concerning conditional marketing authorisations, as it aims at the treatment life-threatening disease, and is designated as an orphan medicinal product.

The product is considered to fulfil the requirements for a conditional marketing authorisation based on the following criteria:

- The benefit-risk balance based on interim phase III data is positive. Zolgensma is designed to address the monogenic root cause of SMA by replacing the defective *SMN1* gene resulting in increased levels of SMN protein.
- Data from the following studies is anticipated to address the uncertainties of an initial CMA based on data from study CL-101 and the interim data of the ongoing phase III studies:
    - Study CL-303, US phase III study investigating the therapeutic IV dose in SMA type 1 patients. Study is completed and full CSR for this study is expected in September 2020.

      The applicant has committed to include in the final CSR of study CL-303:

- ❖ An analysis of cognitive and expressive communication domains of the Bayley scales (change from baseline in raw and scaled scores) should be provided in the final CSR for study CL-303 as an exploratory analysis.
- ❖ A presentation of the normal range of the Bayley scale scores in the graphical representations of the data.
- ❖ The formal analysis of the co-primary endpoints.

- Study CL-302, EU phase III study investigating the therapeutic IV dose in SMA type 1 patients. Full CSR for this study is expected in August 2021.
- Study CL-304. EU phase III study investigating the therapeutic IV dose in pre-symptomatic patients. Full CSR for this study is expected by August 2026.

Data from the ongoing studies should confirm the interim data so far presented by the Applicant with respect to (but not limited to):

- confirmatory end of study survival data
- further achievement and maintenance of motor milestones
- improvements in motor function as measured by CHOP INTEND and Bayley scale scores
- indications of halted disease progression with respect to nutritional and ventilatory support

Experts and caregivers also considered that information in motor and cognitive development is key, and the ongoing studies should help us find a treatment decision model that identifies the most important positive and negative predictors.

- Unmet medical needs will be addressed.

  Although another available treatment option is nusinersen, nusinersen treatment is associated with significant burden for the patient since it requires lifelong intrathecal injection, associated with safety risks. It is agreed that the data from Zolgensma assessed so far strongly suggests that the efficacy of Zolgensma in the intended patient population will exceed that of nusinersen. In addition, it is considered that given the difference in mechanism of action between nusinersen and Zolgensma, Zolgensma is expected to be more efficacious in patients with 2 *SMN2* copies since nusinersen boosts the transcription of full-length protein from the *SMN2* gene. Therefore, a major therapeutic advantage for Zolgensma is to be expected.

From a clinical point of view, the benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

In addition, the CAT considers the following measures necessary to address issues related to safety and efficacy:

In order to further characterise and contextualise the outcomes of patients with a diagnosis of SMA, including long-term safety and efficacy of Zolgensma, the MAH should conduct and submit the results of a prospective observational registry AVXS-101-RG001 according to an agreed protocol.

The applicant should also perform a further evaluation of the finished product specifications when primary and key secondary endpoint data from additional patients with 2 copies of *SMN2* are available (i.e. completion of CL-302 and CL-304 cohort 1). Based on this evaluation, it should be determined whether tightening of the release specification limits is needed to improve consistency of the batches and ensure optimal clinical outcome.

The evaluation should take into account 1) product stability and 2) the correlation between critical quality attributes (genomic titre, infectious titre, *in vitro* relative potency) and clinical outcome in terms of survival, ventilatory support, motor milestones and relevant motor function scores (Bayley) in (pre-)symptomatic subjects born with 2 *SMN2* copies.

The correlation evaluation should include a complete evaluation of the range of FP data against clinical data (e.g. comparing the higher and lower ranges of critical quality attributes) and consider whether batches at the end of shelf life perform as well as batches at the beginning of shelf life. The analysis will take into account known sources of variability in assays and measurement techniques.

The estimated dose given to the patient (vg/kg as determined based on the estimated genomic titre of the product batch at the time of dosing (taking into account stability data) should be included as additional quality attribute.

3.9. Conclusions

The overall B/R of Zolgensma is positive.

The CHMP endorsed the CAT conclusion on Benefit Risk balance as described above.

4. Recommendations

Similarity with authorised orphan medicinal products

The CAT by consensus is of the opinion that Zolgensma is not similar to Spinraza within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

The CHMP endorsed the above CAT conclusion.

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit- risk balance of Zolgensma is favourable in the following indication:

Zolgensma is indicated for the treatment of:

- patients with 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene and a clinical diagnosis of SMA Type 1, or
- patients with 5q SMA with a bi-allelic mutation in the SMN1 gene and up to 3 copies of the SMN2 gene.

Based on the draft opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit- risk balance of Zolgensma in the treatment of the above indication is favourable and therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription.

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached. <Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	**Due date**
Non-interventional post-authorisation efficacy study (PAES): In order to further characterise and contextualise the outcomes of patients with a diagnosis of SMA, including long-term safety and efficacy of Zolgensma, the MAH should conduct and submit the results of a prospective observational registry AVXS-101-RG-001 according to an agreed protocol.	Interim results: - Safety with PSUR, -Efficacy and safety with annual renewal Final results: 2038
The applicant should perform a further evaluation of the finished product specifications when primary and key secondary endpoint data from additional patients with 2 copies of SMN2 are available (i.e. completion of CL-302 and CL-304 cohort 1). Based on this evaluation, it should be determined whether tightening of the release specification limits is needed to improve consistency of the batches and ensure optimal clinical outcome.	Dec 2021 with completion of Study CL-302 and Cohort 1 in Study CL-304

The CHMP endorse the CAT conclusion on the obligation to conduct post-authorisation measures as described above.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14a-4 of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
Post-authorisation efficacy study (PAES): In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients younger than 6 months of age with Spinal Muscular Atrophy Type 1 with One or Two SMN2 Copies the MAH should submit final data on Study AVXS-101-CL-303-CL-303	Final results: at first annual renewal
Post-authorisation efficacy study (PAES): In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients younger than 6 months of age with Spinal Muscular Atrophy Type 1 with One or Two SMN2 Copies the MAH should submit interim and final data on Study Zolgensma-CL-302	Interim results: at each annual renewal Final results: Aug 2021
Post-authorisation efficacy study (PAES): In order to confirm the efficacy and safety and tolerability of a single dose of Zolgensma in patients to genetically diagnosed and pre-symptomatic patients equal or younger than 6 weeks of age at time of treatment with SMA with bi-allelic deletion of *SMN1* with 2 or 3 copies of *SMN2*, the MAH should submit interim and final data on Study Zolgensma-CL-304	Interim results: at each annual renewal Final results: Aug 2026

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the CAT review of the available data, the CAT considers that onasemnogene abeparvovec is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorse the CAT conclusion on the new active substance status claim.