23 April 2026
EMA/CHMP/91326/2026
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Itvisma

International non-proprietary name: Onasemnogene abeparvovec

Procedure No. EMEA/H/C/006498/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AAV	adeno-associated virus
ADRs	Adverse drug reactions
AE	adherent expansion
AEs	adverse events
AESI	adverse events of special interest
CAT	Committee for Advanced Therapies
CHMP	Committee for Medicinal Products for Human Use
CMAP	compound muscle action potential
CMV	cytomegalovirus
CPP	Critical process parameters
CQAs	Critical Quality Attributes
ddPCR	digital droplet Polymerase Chain Reaction
DRG	dorsal root ganglia
EMA	European Medicines Agency
EMG	electromyography
GCP	Good clinical practice
GFP	green fluorescent protein
GLP	Good laboratory practice
GMO	Genetically modified organisms
GMP	Good manufacturing practice
HFMSE	Hammersmith Functional Motor Scale–Expanded
IPCs	in-process controls
ISH	in situ hybridization
IT	intrathecal
ITRs	inverted terminal repeats
IV	Intravenous
KPP	Key process parameters
MFM32	32-item Motor Function Measure
MoA	mechanism of action
MUNE	motor unit number estimation
NCPP	Non-critical process parameters
NKPP	Non-key process parameters
PD	Pharmacodynamics
PIP	paediatric investigation plan
PK	Pharmacokinetics
PNS	Peripheral nervous system
PPs	Process parameters

PPQ	Process Performance Qualification
PRAC	Pharmacovigilance Risk Assessment Committee
PSURs	Periodic safety update reports
RfM	request for modification
RMP	Risk management plan
RoA	routes of administration
SAEs	serious adverse events
SE	suspension expansion
SMA	Spinal muscular atrophy
SMN	Survival Motor Neuron
SmPC	Summary of product characteristics

1. Administrative/regulatory information and recommendations on the procedure

1.1. Protocol assistance

Table 1: Scientific advice and protocol assistance

Date	***Topic (quality / non-clinical/ clinical)***	***Reference number / Coordinator(s)***	***Brief summary of the advice***
22 April 2021	Non-clinical, Clinical	EMA/SA/0000054059	The protocol assistance pertained to the following non-clinical and clinical aspects: •Acceptability of the non-clinical programme to support intrathecal administration for the indication 'treatment of spinal muscular atrophy (SMA) in patients with bi-allelic mutations in the survival motor neuron 1 (SMN1) gene'. •Proposed dose for OAV101 intrathecal administration. •Acceptability of the overall development plan and the proposed randomized controlled phase 3 study design, including objectives, primary analysis, sample size and duration, for registration of the IT formulation for expanded indication for all SMA patients. •Acceptability of the proposed phase 3 trial to assess the safety and efficacy of OAV101 for the proposed indication.
	Quality	EMA/SA/0000062291	The Protocol assistance pertained to the following quality aspects: •Drug product specification and drug product stability specification.
14 September 2023	Quality	EMA/SA/0000141561	The Protocol assistance pertained to the following quality aspects: • The OAV101 (intravenous formulation) active substance analytical comparability approach to register the intrathecal formulation; • the OAV101B (intrathecal formulation) finished product analytical comparability approach following the implementation of the new active substance and the manufacturing facility as a finished product filling site; • the OAV101B finished product stability comparability approach for shelf life.

1.2. Eligibility to the centralised procedure

The applicant Novartis Europharm Limited submitted on 29 April 2025 an application for marketing authorisation to the European Medicines Agency (EMA) for Itvisma (Onasemnogene abeparvovec), through the centralised procedure falling within the Article 3(1) and point 1 of Annex I of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 25 January 2024.

The applicant applied for the following indication: *Itvisma is an adeno-associated virus vector-based gene therapy indicated for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene in patients 6 months of age and older.*

1.3. Legal basis, dossier content and multiples

The legal basis for this application refers to:

Article 8(3) of Directive 2001/83/EC - complete and independent application

The application submitted is

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

This application is submitted as a multiple of Zolgensma authorised on 18/05/2020 in accordance with Article 82(1) of Regulation (EC) No 726/2004.

1.4. Information on paediatrics

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 had been completed.

1.5. Information on orphan market exclusivity

Itvisma was designated as an orphan medicinal product EU/3/15/1509 on 19 June 2015 in the following condition: Treatment of Spinal Muscular Atrophy

1.5.1. Similarity with authorised orphan medicinal products

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 from the start of the procedure {Spinraza (nusinersen)}.

1.6. Applicant's request(s) for consideration

1.6.1. Accelerated assessment request

The applicant requested accelerated assessment in accordance to Article 14(9) of Regulation (EC) No 726/2004. The CHMP did not agree to the applicant's request for an accelerated assessment as discussion was anticipated at the MAA.

1.7. Third party interventions

A third party intervention was received during the procedure. Discussion on this is reflected as relevant in the scientific report below in efficacy/safety discussions and/or benefit/risk.

1.8. Steps taken for the assessment of the product

The rapporteur and co-rapporteur appointed by the CAT/CHMP were:

CAT Rapporteur:	Emmely de Vries
CAT Co-rapporteur:	Jan Mueller-Berghaus

The application was received by the EMA on	29 April 2025
The procedure started on	22 May 2025
The CAT rapporteur's first assessment report was received on	11 August 2025
The CAT Co-rapporteur's first assessment report was added to the rapporteur's report on	13 August 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	4 September 2025
The CHMP agreed on the consolidated list of questions (LoQ) to be sent to the applicant during the meeting on	12 September 2025
The applicant submitted the responses to the CHMP consolidated List of Questions on	18 December 2025
The CAT rapporteur circulated the CAT and PRAC rapporteurs joint assessment report on the applican responses to the list of questions (LoQ) to all CHMP and PRAC members on	2 February 2026
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 February 2026
The CHMP agreed on a list of outstanding issues (LoOI) to be sent to the applicant on	20 February 2026
The applicant submitted the responses to the CHMP list of outstanding issues on	16 March 2026
The CAT rapporteur circulated the CAT and PRAC rapporteurs Joint assessment report on the applicant's responses to the list of outstanding issues to all CHMP and PRAC members on	14 April 2026
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 Itvisma on	23 April 2026
The CHMP adopted a report on similarity of Itvisma with Spinraza authorised from the start of the procedure on (see appendix on similarity)	23 April 2026

1.9. Final CHMP outcome

1.9.1. Considerations related to paediatrics

The requirements for the submitted dossier in relation to paediatrics are described in section 1.5 of this report.

The CHMP reviewed the available paediatric data of studies subject to the agreed paediatric investigation plan (PIP) P/0162/2019 and the results of these studies are reflected in the summary of product

characteristics (SmPC) and, as appropriate, the package leaflet.

1.9.2. Considerations related to orphan market exclusivity

The requirements of the submitted dossier in relation to orphan market exclusivity are described in section 1.6 of this report.

1.9.2.1. Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Itvisma is not similar to Spinraza within the meaning of Article 3 of Commission Regulation (EC) No 847/2000. See the appendix on similarity.

1.9.3. Final opinion

Based on the CAT review of data on quality, safety and efficacy, the CHMP considers that the benefit-risk balance of Itvisma is favourable in the following indication(s):

Itvisma is indicated for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the *SMN1* gene in patients 2 years of age and older.

The CHMP, therefore, endorses the CAT conclusion and recommends the granting of the marketing authorisation subject to the conditions described in the following sections.

1.9.4. Other conditions and requirements of the marketing authorisation

1.9.4.1. 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.

1.9.5. Conditions or restrictions with regard to the safe and effective use of the medicinal product

1.9.5.1. Risk management plan (RMP)

The marketing authorisation holder (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.

1.9.5.2. Additional risk minimisation measures

Prior to use of Itvisma in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority (NCA).

The MAH shall ensure that in each Member State (MS) where Itvisma is marketed, healthcare professionals (HCP) who are expected to prescribe, dispense and administer Itvisma are provided with the following Healthcare Professional Information Pack:

- SmPC
- Healthcare professional guide

The healthcare professional guide shall contain the following key messages:

- Before the start of treatment:
  - Evaluate the patient's vaccination schedule;
  - Inform the caregiver(s) about the main risks with Itvisma and their signs and symptoms, including hepatotoxicity, thrombocytopenia, peripheral sensory neuropathy and TMA; about the need for regular blood sampling; about the importance of corticosteroids;
  - Inform the caregiver(s) of the need for increased vigilance in the prevention, monitoring, and management of infection before and after Itvisma administration;
  - Patients should be tested for the presence of AAV9 antibodies.

- At the time of treatment:
  - Check if the overall health status of the patient is suitable for administration of Itvisma (e.g. resolution of infections) or if a postponement is warranted;
  - Check that corticosteroid treatment was started before administration of Itvisma.
- After treatment:
  - The corticosteroid treatment should continue for at least 2 months and not be tapered until AST/ALT are less than 2 x ULN, and all other assessments, e.g. total bilirubin, return to normal range;
  - Close and regular monitoring (clinical and laboratory) of the individual patient course should be performed for at least 3 months;
  - Prompt assessment of patients with worsening liver function tests and/or signs or symptoms of acute illness;
  - If patients do not respond adequately to corticosteroids, or if liver injury is suspected, HCP should consult a gastroenterologist or hepatologist;
  - If TMA is suspected, a haematologist and/or nephrologist should be consulted.
- Patients and caregivers should be informed about the signs and symptoms of peripheral sensory neuropathy and be advised to notify their physician promptly if such symptoms occur. If peripheral sensory neuropathy is suspected, complete neurological evaluation and other testing and/or symptom management should be considered based on the patient's clinical presentation

The MAH shall ensure that in each Member State (MS) where Itvisma is marketed, all caregivers of patients in whom Itvisma treatment is planned or who have received Itvisma are provided with the following Patient Information Pack:

- Package Leaflet
- Patient/patient's caregiver information guide

The patient/patient's caregiver information guide shall contain the following key messages:

- What is SMA.
- What is Itvisma and how it works.
- Understanding the risks of Itvisma.
- Treatment with Itvisma: important information before, on the day of treatment and after treatment, including when to seek medical attention.
- It is recommended that patients present an adequate overall health status (e.g. hydration and nutritional status, absence of infection) prior to Itvisma treatment, otherwise treatment may need to be postponed.
- Itvisma may increase the risk of abnormal clotting of blood in small blood vessels (thrombotic microangiopathy). Thrombotic microangiopathy is serious and may lead to death. Tell your or your child's doctor immediately if you notice signs and symptoms such as bruising, seizures or decrease in urine output. You or your child will have a regular blood test to check any decrease of platelets, the cells responsible for clotting, at least weekly for the first month after treatment. Depending on the values and other signs and symptoms, further evaluations may be required.
- Itvisma can lower blood-platelet counts (thrombocytopenia). Cases generally occurred within the first week after the patient was given Itvisma. Possible signs of a low blood-platelet count you need to look out for after you or your child is given Itvisma include abnormal bruising or bleeding. Speak to your or your child's doctor if you see signs such as bruising or bleeding for longer than usual if you or your child has been hurt.
- Itvisma can lead to an increase in enzymes (proteins found within the body) produced by the liver. In some cases, Itvisma can affect the function of the liver and lead to injury of the liver. Possible signs you need to look out for after you or your child is given this medicine include vomiting, jaundice (yellowing of the skin or of the whites of the eyes), or reduced alertness. Tell your or your child's doctor straight away if you notice any symptoms suggestive of injury to the liver. You or your child will have a blood test to check how well the liver is working before starting treatment with Itvisma. You or your child will also have regular blood tests for at least 3 months after treatment to monitor for increases in liver enzymes. Depending on the values and other signs and symptoms, further evaluations may be required.
- You or your child will be given a corticosteroid medicine such as prednisolone before treatment with Itvisma, and for about 2 months or longer after Itvisma treatment. The corticosteroid medicine will help manage effects of Itvisma such as increase in liver enzymes that you or your child could develop after treatment with Itvisma.
- Tell your or your child's doctor in the event of vomiting before or after treatment with Itvisma, to make sure that you or your child does not miss corticosteroid dosing.
- Before and after treatment with Itvisma it is important to prevent infections by avoiding situations that may increase the risk of the patient getting infections. Caregivers and close contacts with the patient should follow infection prevention practices (e.g. hand hygiene, coughing/sneezing etiquette, limiting potential contacts). Inform the doctor straight away in case of signs and symptoms suggestive of infection, such as respiratory infection (coughing, wheezing, sneezing, runny nose, sore throat or fever) prior to administration as the administration may need to be delayed until the infection is resolved, or after treatment with Itvisma as it may lead to medical complications that may require urgent medical attention.
- Tell your or your child's doctor immediately if you or your child experience numbness, tingling, prickling or pain in the arms, hands, legs and/or feet after being given Itvisma. These may be signs of peripheral sensory neuropathy
- Useful further information (supportive care, local associations).
- Contacts of the physician/prescriber.

1.9.6. Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

1.9.7. Proposed list of recommendations

Table 2: Proposed list of recommendations

Description of recommendation(s)
Quality: The applicant commits to transferring the remaining finished product release methods to their EU testing facilities.
Quality: The applicant commits to revising the WCB qualification protocol for future cell banks to include a test for MVM.
Quality: The applicant commits to introducing pressure monitoring for sterile filtration
Quality: The applicant commits to re-analysing the total protein acceptance criterion for finished product release once 30 OAV101B batches have been manufactured and the corresponding analytical data are available.
Quality: The applicant commits to updating aseptic process simulation (APS) approach to include the demonstration of representative containers and worst-case processes are captured every 6 months.
Quality: The applicant commits to the development, validation, and implementation of an independent, direct capsid titer determination method suitable for finished product release specification
Quality: The applicant commits to including in the dossier, the validation data demonstrating that the finished product vial sterilization process achieves the required sterility assurance level (SAL).

2. Introduction

2.1. Therapeutic context

Spinal muscular atrophy

SMA is an autosomal-recessive neuromuscular disease affecting approximately 1:10,000 live births globally, with an estimated incidence in Europe ranging from 1 in 3,900–16,000 live births (Verhaart et al 2017). Prior to disease-modifying treatments, SMA was the leading cause of infant mortality due to genetic disease (Sugarman et al 2012, Awano et al 2014).

SMA is caused by bi-allelic loss of SMN1 gene on chromosome 5q13, which leads to reduced Survival Motor Neuron (SMN) protein. Depletion of the SMN protein produces degeneration of motor neurons in the brainstem and anterior horn of the spinal cord, leading to progressive skeletal muscles atrophy. A small amount of the SMN protein is also produced by the *SMN2* gene, an almost identical but partially functional centromeric copy. When *SMN1* is missing/mutated, the only source of SMN protein is *SMN2*.

Age of symptoms onset in SMA correlates with the degree to which motor function is affected, whereby the earlier the onset, the greater the deficits. Accordingly, SMA has been historically classified into 4 clinical subtypes, based on age of onset and maximal motor function achieved without treatment (Table 3). SMA Type 1 is the most common and a severe form, representing 45-60% of the cases, and whose prognosis without treatment is particularly dire: symptoms manifest soon after birth (<6 months of age), children never gain the ability to sit, and typically do not survive past age 2 years. SMA Type 2 (~20-30% of cases) manifests before 18 months, and children are able to maintain sitting unassisted but never walk independently. SMA type 3 patients (~10-20% of cases) attain independent walking, but

may eventually lose this ability (type 3a has onset between 18-36 months; type 3b has onset >3 years). SMA type 4 (<5% of cases) is an adult onset disease. In addition, SMA type 0 describes a rare and most severe form with prenatal onset (Kolb and Kissel 2011).

Disease severity in SMA relates to the amount of neuronal SMN protein, which in turn primarily depends on the number of the *SMN2* gene copies (usually between 1 to 5), each producing a proportion of the functional SMN protein. Patients with bi-allelic *SMN1* deletion/mutation and up to 2 copies of *SMN2* have high probability of developing SMA type 1 (99% with 1 copy, 97% with 2 copies). Patients with 3 *SMN2* copies have a 83% risk of developing SMA type 2, and patients with 4 SMN2 copies have a 84% risk of developing SMA type 3. Hence, although other factors (as genetic modifiers) can influence the SMA phenotype, the *SMN2* copy number has an important prognostic value (Feldkötter et al., 2002).

The combination of genotypic and phenotypic characteristics led to the subdivision in SMA types as presented in the table below (source: zolgensma-epar-public-assessment-report_en, adapted from Kolb 2011).

Table 3: 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.

Together, the different SMA types constitute a spectrum, ranging from early and severe forms, to mild forms manifesting later in life. As constructed prior the availability of effective treatment, the classification into subtypes provided characterization of the natural history, and anticipatory guidance to caregivers/patients.

With the advent of disease-modifying therapies, and the resulting different clinical picture of the treated patients, a functional classification based on the maximum motor function achieved can be used instead: 'Non-sitters' with individuals who are unable to sit independently and may require support for trunk control, 'Sitters' with individuals who can sit independently but are unable to walk and 'Walkers' with individuals who demonstrate the ability to walk independently.

When SMA is suspected clinically or from family history, the diagnosis is confirmed by genetic testing. Several countries in EU have adopted SMA in their newborn screening programmes.

Therapies currently used in the EU/EEA

In the EU/EEA, the available therapeutic options for SMA are SMN gene replacement therapy with onasemnogene abeparvovec administered intravenously (Zolgensma), and splicing modulators acting on the SMN2 gene, for which two products are available: nusinersen (Spinraza) and risdiplam (Evrysdi).

Zolgensma (onasemnogene abeparvovec IV) was approved by the CHMP in 2020, for the treatment of patients with 5q 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 SMN2 gene.

Zolgesma contains the same active substance of Itvisma, but is designed as a one-time intravenous (IV) injection at the recommended dose of $1.1x10^{14}$ vg per kg bodyweight.

Spinraza (nusinersen) was approved by the CHMP in 2017, for the treatment of 5q SMA. Nusinersen is an antisense oligonucleotide that blocks a splice silencer within intron 7 of SMN2 pre-mRNA, thus increasing the production of full-length mRNA and SMN protein levels. Nusinersen showed clinical benefit in children aged 2 to 9 years with SMA who had symptom onset after 6 months of age (later-onset SMA), as assessed by the SMA-specific Hammersmith Functional Motor Scale–Expanded (HFMSE) (Mercuri et al 2018). Nusinersen is administered intrathecally, starting with 4 doses during a 2-month loading period followed by maintenance dosing every 4 months. The approved 12-mg dose for nusinersen is the same for all age and weight groups (Spinraza SmPC). Nusinersen requires lifelong intrathecal injections, which is associated with safety risks.

Evrysdi (risdiplam) was approved by the CHMP in 2021, for the treatment of 5q SMA in patients with a clinical diagnosis of SMA Type 1, Type 2 or Type 3 or with one to four SMN2 copies. Risdiplam is a small molecule that facilitates SMN2 exon 7 retention within the mRNA and thus increases SMN protein levels. Risdiplam treatment resulted in improvements in motor function in patients with later-onset SMA aged 2 to 25 years based on the 32-item Motor Function Measure (MFM32). Risdiplam is administered orally. Dosing is weight-based up to 20 kg and a flat dose over 2 years of age and 20 kg (Evrysdi SmPC).

In addition, care for SMA patients commonly involves physical therapy, occupational therapy, and the use of adaptive equipment, aiming at maximizing patient's functional capacity and quality of life.

Due to the progressive nature of SMA, early diagnosis and treatment is crucial. The available therapies can improve survival of motor neurons, but cannot restore motor neurons that have being lost. Hence, treatment outcomes are highly dependent on when they are initiated.

2.2. Aspects of development

The clinical development program for onasemnogene abeparvovec for intrathecal (IT) administration (referred to herein as OAV101B) consists of:

- one pivotal Phase III randomized, sham-controlled, double-blind global, multi-center study in treatment-naïve patients 2 to <18 years of age (COAV101B12301 or **B12301**),
- one Phase I/II open-label safety and efficacy study in treatment-naïve patients 6 to <60 months of age (AVXS-CL-102 or **CL-102**),
- one supporting Phase IIIb open-label safety study in treatment experienced patients 2 to <18 years of age (COAV101B12302 or **B12302**).

In addition, patients from these three studies were invited to enroll into the long-term follow-up studies AVXS-LT-002 (**LT-002**) or COAV101A12308 (**A12308**).

2.2.1. Protocol assistance

The Applicant received written protocol assistance on the development of OAV101B for the treatment of SMA from the CHMP on 22/04/2021 *(*EMA/SA/0000054059). The protocol assistance pertained to the following non-clinical, clinical, aspects:

Non-clinical:

- Acceptability of the non-clinical programme to support intrathecal administration in the treatment of SMA for the indication 'treatment of spinal muscular atrophy (SMA) in patients with bi-allelic mutations in the survival motor neuron 1 (SMN1) gene'.

Clinical:

- Proposed dose for OAV101 intrathecal administration.
- Acceptability of the overall development plan and the proposed randomized controlled phase 3 study design, including objectives, primary analysis, sample size and duration, for registration of the IT formulation for expanded indication for all SMA patients.
- Acceptability of the proposed phase 3 trial to assess the safety and efficacy of OAV101 for the proposed indication.

The CHMP endorsed the design of the pivotal study B12301 (randomized, double-blind, sham-control) to assess the efficacy and safety of OAV101B in a heterogenous SMA population. The study population, the sample size, efficacy endpoints, statistical methodology, and study duration were considered acceptable.

A paediatric investigation plan (PIP) was agreed upon with the EMA/PDCO on 14 August 2018, including a deferral, for the treatment of SMA. The PIP was later amended, with the latest request for modification (RfM) approved on May 6, 2024. Positive opinion confirmed, dated 25 April 2025, on the full compliance check (procedure EMA/PE/0000253277) confirming compliance with all the agreed PIP measures.

2.3. Description of the product

OAV101B (onasemnogene abeparvovec for intrathecal administration) is an adeno-associated virus (AAV) vector-based gene therapy given as a one-time intrathecal injection.

The mechanism of action of onasemnogene abeparvovec is delivery of SMN complementary DNA encoding a functional, full-length SMN protein into target cells. The vector genome persists in cells, as episomal monomeric and concatemeric circles in a structure consistent with that of chromatin, resulting in supply of the SMN protein.

The proposed therapeutic indication was initially "*Itvisma is an adeno-associated virus vector-based gene therapy indicated for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene in patients 6 months of age and older*".

OAV101B is administered intrathecally via lumbar puncture, at the recommended dose of $1.2x10^{14}$ vg.

OAV101B has been approved on 24 Nov 2025 by the FDA under the proprietary name ITVISMA for the indication "*Treatment of spinal muscular atrophy (SMA) in adult and pediatric patients 2 years of age and older with confirmed mutations in survival motor neuron 1 (SMN1) gene*.".

On 27 Nov 2025, the Applicant informed that the same invented name (Itvisma) will be used for the MAA of OAV101B, for the purpose of harmonization. The names OAV101B and Itvisma will be used interchangeably in this report.

The IV formulation of onasemnogene abeparvovec (Zolgensma) has been approved in more than 55 countries, including the EU/EEA, US, and Japan. In the EU/EEA, Zolgensma has been authorized for treatment of patients with clinical diagnosis of SMA type 1, or patients with a bi-allelic mutation in the SMN1 gene and up to 3 copies of the SMN2 gene.

2.4. Inspection issues

GCP ispections

Four Health Authority site inspections were conducted for study B12301, and specifically:

- 2 GCP Routine inspections conducted by the Egyptian Drug Authority;
- 1 GCP Routine inspection conducted by the Medicines Control Council (MCC);
- 1 GCP Routine inspection conducted by the Ministry Inspectorate, Ministry of Health, Vietnam.

Full reports of these GCP inspections were provided by the Applicant, no critical issues were found in any of these inspections.

A routine EMA GCP inspection was conducted by the Health and Youth Care Inspectorate and the Danish Medicines Agency in the South African site, the Vietnam site and the sponsor site of study B12301. The outcome of this inspection has been provided (EMA/IN/0000279992, dated 16 Jan 2026).

At the EMA inspection of the investigator site in South Africa, there were 8 major findings (no critical findings). At the EMA inspection of the investigator site in Vietnam there were 12 findings, 10 major and 2 minor (no critical findings).

At the EMA inspection of the sponsor site, Novartis AG (Basel, Switzerland), there were 14 findings, **4 critical findings** and 10 major findings. These are discussed later in the report.

3. Quality aspects

3.1. Introduction

Itvisma is a gene therapy medicinal product containing onasemnogene abeparvovec as active substance (AS). Onasemnogene abeparvovec is produced in human embryonic kidney cells by recombinant DNA technology.

The finished product (FP) is presented as a solution for intrathecal injection containing 4 x 10^{13} vector genomes (vg)/mL of onasemnogene abeparovec. Each single-dose vial contains 1.2 × 10^{14} vector genomes (vg) of onasemnogene abeparvovec in 3 mL of solution.

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

The product is available in a 5 mL single-dose cyclo-olefin polymer vial with a 20 mm chlorobutyl rubber stopper and an aluminum flip-off seal with a coloured plastic cap. Each vial has a fill volume of not less than 3 mL.

The legal basis of this marketing authorisation application (MAA) is Article 8.3 of Directive 2001/83/EC as amended - complete and independent application. A full Module 3 was provided and all the data has been assessed accordingly. References to the already approved Zolgensma (EMEA/H/C/004750) containing the same active substance are just made for comparison purposes.

3.2. Active substance

3.2.1. General information

Itvisma (company code: OAV101) is a non-replicating, recombinant adeno-associated virus serotype 9

(AAV9) containing the human survival motor neuron (SMN) gene under the control of the cytomegalovirus (CMV) enhancer/chicken-β-actin-hybrid promoter (CB). One of the AAVs 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 is a clear, to slightly opaque, colourless to faint white solution at a concentration of 3.4 to 7.3 x 1013 vg-containing particles per millilitre.

3.2.2. Manufacture, characterisation, and process controls

Description of manufacturing process and process controls

The active substance will be produced at Novartis Gene Therapies in Durham, USA. The site is covered by a valid GMP certificate. All the manufacturing, testing testing and storage facilities for the active substance are covered by valid GMP certificates.

A sufficiently detailed manufacturing process description has been provided. The OAV101 active substance process is the same as the manufacturing process of the active substance of the licensed product Zolgensma.

Briefly, for commercial Process 2.0 it consist of working cell bank (WCB) vial thaw, cell expansion, bioreactor inoculation, transfection with triple DNA plasmid solution, endonuclease treatment, cell lysis and harvest, harvest clarification, exchange chromatography, ultra-diafiltration, ultracentrifugation, UF/DF, filtration and filling.
A general overview and flow chart of the upstream and downstream manufacturing process is provided, as well as detail descriptions of the different process steps with flow diagrams including process inputs and in-process controls (IPCs). The batch number is described. The batch size is defined by the starting material. The WCB is thawed and expanded to provide sufficient cells to inoculate the bioreactor.

Reprocessing has not been described.

Tabular overviews of key and critical process parameter (CPP) ranges and IPCs with acceptance criteria are provided in S.2.4. The selection process parameters and IPCs is in line with expectations. One intermediate is defined in the manufacturing process.Tests and specifications for this intermediate are acceptable. Stability has been demonstrated

Control of materials

The starting materials for onasemnogene abeparvovec consist of a HEK293 cell bank and three recombinant plasmids.

The history of the HEK293 cell banks used is sufficiently described. A pre-master cell bank (MCB) was established in 2015 from a vial of HEK293 cells from ATCC and used for manufacture of the MCB in 2016. The MCB was tested in line with ICH Q5A. Four WCBs have been manufactured from the MCB. Acceptance criteria and release test results are provided, including test results from an extended cell bank, and do not give rise to questions. Cell bank stability is monitored. The protocol for introduction of future cell banks is acceptable.

Three plasmids are used for the manufacture of OAV101: pSMN, pAAV2/9 and pHelp. For each plasmid a schematic map is provided. An overview of the sites involved in plasmid manufacturing and testing is provided. Cell banks used for the manufacture of the plasmids are tested in line with Ph. Eur. 5.34. The release specifications for the plasmids are acceptable. Plasmid stability has been demonstrated.

Raw materials of human, animal or recombinant origin used in the active substance manufacturing process include irradiated foetal bovine serum (FBS), media (contains human transferrin and recombinant human insulin from yeast), and endonuclease. Specifications for these materials are provided. Virus and TSE safety of these materials is sufficiently addressed.

For the buffers and media tabular overviews are provided, including the composition, specifications, supplier and process step in which the buffer/medium is used. The applicant provided a commitment to include the qualitative composition of the commercially derived media in the dossier (**REC 1**). Agreements with the suppliers to notify the applicant in case of changes to the medium are in place.

Process validation

The commercial manufacturing process (Process 2.0, suspension expansion (SE)) is based on Process 2.0, adherent expansion (AE). Process Performance Qualification (PPQ) was performed for Process 2.0 AE using 3 consecutive process performance qualification (PPQ) runs for each stage of the process. The PPQ study for Process 2.0 SE only included the cell expansion stage and intermediate manufacture, as the other parts of the manufacturing remained the same as Process 2.0 AE.

With the exception of two minor deviations that were appropriately analysed and discussed, all validation acceptance criteria were met. The results of the PPQ studies demonstrate that the process can consistently operate within the limits defined in S.2.2 and yields active substance that meets its specification. The PPQ studies do not give rise to questions. Column re-use and shipping have also been appropriately validated.

Manufacturing process development

Four different active substance manufacturing processes have been used during development: Process A, Process 1.0 (previously called Process B), Process 2.0 AE, and Process 2.0 SE. A detailed description of the development of Process 1.0 is provided, including the approach used for process parameter risk assessment, process parameter classification, and process characterisation studies. The provided information is sufficient. The changes that were introduced to the process and control strategy with the introduction of Process 2.0 have been discussed and justified in the supportive comparability evaluations.

Process A has been used to manufacture the batch used for the Phase 1 clinical study. A separate document describing the development of the manufacturing process and control strategy from Process A to Process B (Process 1.0), including assessment of comparability, is included in the dossier. Previously it was concluded that the available data did not allow to draw conclusions on comparability between the Process A batch and Process B batches.

Process 1.0 was used to manufacture batches used in the toxicology study, the Phase 1 clinical trial (CL-102, STRONG) and the pivotal Phase III clinical trial(STEER), and Process 2.0 SE batches in the supportive Phase III clinical trial (STRENGTH) and a reproductive toxicology study. Comparability of Process 2.0 AE and Process 1.0 was evaluated using data from, respectively, 123 and 115 Process 1.0 active substance and finished product batches (including corresponding cell culture batches and CEX intermediates) and 3 Process 2.0 AE active substance and finished product batches (including corresponding cell culture batches and CEX intermediates). The comparability assessment included process comparability, analytical comparability (in-process, release and additional characterisation testing) and stability. The provided data support comparability between Process 1.0 and Process 2.0 AE. Process 2.0 AE batches have not been used to manufacture clinical batches.

The only difference between the commercial Process 2.0 SE and Process 2.0 AE is the cell expansion process. Comparability was evaluated using Process 2.0 AE batches and Process 2.0 SE batch, including corresponding cell culture batches and intermediates. The comparability assessment included process comparability, analytical comparability (in-process, intermediate, and release testing) and stability. The provided data support comparability between Process 2.0 AE and Process 2.0 SE. Process 2.0 SE will be the commercial manufacturing process.

A separate document is provided describing the comparability assessment between OAV101B IT (Intrathecal) batches used for the OAV101B clinical trials and OAV101B IT batches intended for commercial distribution. The study included analytical comparability evaluation of Process 1.0 AS and FP lots used in the STRONG and STEER clinical trial, AS lots manufactured using Process 2.0 SE, Process 2.0 FP lots used in the STRENGTH clinical trial, Process 2.0 FP PPQ lots and Zolgensma FP lots. The provided data support comparability of the clinical and commercial batches.

Characterisation

The tests performed to elucidate the properties of the active substance included aggregation by size exclusion chromatography (SEC), protein profile by capillary electrophoresis sodium dodecyl sulfate (CE-SDS), genome sequence by next generation sequencing (NGS), and vector structure and integrity by CryoEM. In addition, the caesium chloride (CsCl) gradient bands were characterised by genomic titre, CE-SDS, southern blot, potency, residual DNA, and sedimentation velocity analytical ultracentrifugation tests. The provided information is sufficient.

The applicant identified process-derived impurities. The AS release specification for process-derived impurities. For the other impurities it is sufficiently justified that no release testing is in place.

Product-related impurities are not discussed by the applicant as they are discussed at finished product level. Information on aggregates and empty capsids is, however, provided as part of characterisation. Capsid distribution (empty, full, other peaks), genomic integrity, by NGS and total purity and impurities by CE-SDS are tested as part of FP release. This is considered sufficient.

3.2.3. Specification

The proposed active substance release test panel and acceptance criteria are acceptable. The active substance is tested for pH, appearance, osmolality, titre, identity, impurities, and microbiological attributes. The acceptance criteria are defined based on Process 1.0 active substance batches, which can be accepted as comparability between Process 1.0 and Process 2.0 has been demonstrated. The deviation of the active substance release specifications from Ph. Eur. monograph 3186 Gene Therapy Medicinal Products For Human Use has been sufficiently justified.

Analytical procedures and reference standards

The description of the analytical procedures includes information on method principle, equipment and materials, preparation of standards and controls, method procedure, system suitability criteria, and sample acceptance criteria. The provided information is sufficient. The results of the method validations do not give rise to questions.

Batch analysis

Batch analysis data are provided for a total of 6 at scale batches encompassing Process 1.0 and Process 2.0 batches. Batch results are generally consistent and indicate that the process is under control.

Container closure

The OAV101 active substance is filled into 250 mL PETG bottles. The choice of container is acceptable, and sufficient information is provided on compatibility, extractable and leachables, and sterility. The container material, dimensions and specifications are provided. Sterilisation of the container is performed by gamma irradiation.

Reference materials

Due to the similarity in formulations between OAV101 active substance and OAV101 Finished Product, a finished product reference material is employed for testing both the active substance and finished product, as described in finished product section.

A two-tiered in-house reference standard system is in place. The reference standards, stability test plan, and qualification protocol for future reference standards are the same as for the applicant's registered product Zolgensma, which contains the same active substance. This is acceptable. Sufficient information on the reference standards is included in the CTD.

3.2.4. Stability

The OAV101 Process 2.0 AS stability studies were designed to evaluate three storage conditions.

The OAV101 Process 2.0 AS stability samples are stored in vials representative of the final OAV101 AS container closure system described in Module 3.2.S.6.

Currently, lots from Process 2.0 AE AS and from Process 2.0 SE AS support the stability of the OAV101 Process 2.0. The lots were placed on stability at long-term, accelerated, and stressed storage conditions with testing attributes to evaluate the physicochemical properties (appearance, pH, Osmolality), quantity/strength, purity and potency. The accelerated and stressed studies are also complete. All stability data are provided in Module 3.2.S.7.3. Stability data for OAV101 Process 2.0 AS is within expectation and no adverse trends have been identified.

The statistical comparability trend analysis performed on the accelerated stability data demonstrated that the stability profiles of P1.0 AS and P2.0 AE and SE AS batches are comparable, which enabled to predict the stability of P2.0 AE/SE AS and assign a common shelf life. In addition, long-term real time data was collected to confirm the appropriateness of assigning a common shelf life to P2.0 AE and P2.0 SE AS.

The statistical comparability analysis demonstrated that the OAV101 Process 2.0 AE AS stability is not statistically different from the OAV101 Process 1.0 AS stability.

The provided stability data support the proposed shelf life.

3.3. Finished medicinal product

3.3.1. Description of the product and pharmaceutical development

Description of the product

The finished product is presented as a solution for intrathecal injection containing 4 x 10^{13} vg/mL of onasemnogene abeparovec, a non-replicating, recombinant AAV9 containing the human SMN gene. The active product is formulated in 20 mM Tromethamine, 1 mM Magnesium Chloride, 200 mM Sodium Chloride, and 0.005 % w/v Poloxamer 188. The product is available in a 5mL cyclo-olefin polymer vial with a 20 mm chlorobutyl rubber stopper and a aluminum, flip-off seal with a colored plastic cap. Each

vial has a fill volume of at least 3 mL. All excipients are well known pharmaceutical ingredients, and their quality is compliant with Ph. Eur standards.

Pharmaceutical development

Itvisma has the same formulation composition as the applicant's registered product Zolgensma, but the virus vector is formulated at a higher concentration. The choice of excipients is sufficiently justified. Stability of the active substance in the proposed formulation buffer has been demonstrated. The choice of container closure system is supported by container closure integrity testing, studies to evaluate the impact of freeze-thaw, migration studies for the label adhesive and inks, and extractable/leachables studies.

The SmPC defines the administration device components that are compatible with Itvisma. Two studies to demonstrate compatibility with the defined devices have been performed. The first study evaluated the impact on sub-visible and visible particulates using formulation buffer as no additional particulate was expected to be generated as a result of the vector capsid. In the second compatibility study the impact on vector recovery and potency was evaluated. Overall, the provided data support the use of administration devices and in-use stability as defined in the SmpC.

The applicant has provided an overview of the control strategy. Critical Quality Attributes (CQAs) were identified based on severity and uncertainty scoring. The selection of CQAs is acceptable. All CQAs are controlled by in-process or release testing. Process parameters (PPs) were defined as critical (CPP), non-critical (NCPP), key (KPP) and non-key (NKPP) based on risk and impact of failure. Definitions of the different PP categories is provided in the CTD. The definition of CPP is in line with the ICH definition. A tabular overview of CPPs and KPPs as well as in-process controls (IPCs) and critical IPCs is provided. The selection of CPPs and KPPs is in general in line with expectations; the applicant committed to include a control for filtration pressure (**REC 2**).

Two different finished product manufacturing processes have been used during clinical development: Process 1 and Process 2. Initially, the finished product was manufactured at GTxLV (Novartis Gene Therapies, Inc. Libertyville, IL, USA), using either Process 1 or Process 2. The commercial manufacturing process and site will be Process 2 and GTxNC.

Process 1 has been used been used at GTxLV for manufacture of clinical batches used in the STRONG study (CL-102). The active substance batches used for the manufacture of these clinical batches were manufactured using active substance Process 1.0. Process 2 has been used at GTxLV to manufacture the development batches and clinical batches used in the STEER study. Comparability of Process 1 and 2 at GTxLV was evaluated using Process 1 and Process 2 (PPQ) batches. The study included a comparison of IPC and process parameter results and release test results. The provided data support comparability.

Process 2 was transferred from GTxLV to the proposed commercial manufacturing site GTxNC, with minor differences in process parameters and controls. In addition, the active substance batches used for the manufacture of Finished Product at GTxLV were manufactured using active substance Process 1.0, whereas at GTxNC active substance batches manufactured using active substance Process 2.0 were used. Process 2 has been used at GTxNC to manufacture clinical batches used in the STRENGTH study, PPQ batches and commercial batches. Comparability of Process 2 at GTxLV and GTxNC was evaluated using GTxLV and GTxNC batches. The study included a comparison of IPC and process parameter results and release test results. The results of the GTxNC batch were in general within the range of the results of the GTxLV batches. The additional batch analysis data available in P.5.4 further support comparability.

Overall, comparability during development has been sufficiently demonstrated.

3.3.2. Manufacture of the product and process controls

Manufacture

The finished product will be manufactured at Novartis Gene Therapies, USA. Prove of GMP compliance has been provided.

A sufficiently detailed manufacturing process description has been provided, including a flow chart, tabular overviews of key and critical process parameters, set-points and operational ranges, and information on in-process controls and hold times. Briefly, the OAV101 finished product process consists of active substance pooling, sterile filtration and concentration adjustment, filling, visual inspection, labelling and secondary packaging. Reprocessing of the sterile filtration step may be performed and is supported by the PPQ study. The conditions for reprocessing are described.

The proposed operational ranges for the process parameters and hold times are mostly agreed.

Process controls

The selection of in process controls is acceptable and includes control for genomic titre, filter integrity, fill weight, visual inspection and container closure integrity. The limits for pre- and post-use filter integrity testing are supported by validation data.

For bioburden testing prior to sterile filtration, a sample volume of 10mL will be collected. This volume is in lieu of the 100 mL sample volume that is required by the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container (EMA/CHMP/CVMP/QWP/ 850374/2015), considering 1% of the maximum validated batch size is less than volume provided in the guidance.

A justification has been provided that the probability of detecting microbial contamination is sufficient to guarantee a sterile product.

Process validation / verification

Process Performance Qualification (PPQ) was performed using 3 consecutive commercial scale PPQ runs. The results of the PPQ study demonstrates that the process can consistently operate within the limits defined in P.3.3 and yields Finished Product that meets its specification. All acceptance criteria for process parameters, in process controls and FP release tests were met. Additional process validation performed, included re-filtration and hold-time validation as part of the PPQ, validation of the sterilisation process, aseptic process validation, filler qualification and shipping validation. Overall, the provided validation studies sufficiently support the proposed manufacturing process.

3.3.3. Product specification

Finished product is tested for pH, appearance, osmolality, sub-visible particles, fill weight, quantity, potency, identity. Impurity, container clousure integrity and microbiological attributes.

The potency assay consists of an *in vitro* Relative Potency cell-based assay. The cells used in this assay are derived from mouse primary neural progenitor cells (NPCs) that are isolated from the cortex of SMNΔ7 mice. SMNΔ7 mice are an in vivo animal model of SMA disease containing homozygote allele of SMN1 gene knockout and homozygote allele of SMN2 with deletion of its exon 7, leading to no expression of SMN protein in cells. The cell model system used in this assay is the terminally differentiated NPCs (mTD-NPC-Δ7). In mTD-NPC-Δ7 transduced with increasing doses of the medicine, increasing levels of SMN protein expression is measured by staining using a monoclonal antibody specific to SMN.

The proposed finished product release and stability test panel is acceptable. The applicant committed to include the control for fill weight that is performed in-process. The absence of tests for vector aggregates, infectious particle titre, ratio of viral genome titre to infectious particle titre, and expression from the genetic insert is sufficiently justified. A commitment was provided to include an independent, direct capsid titre determination method in the finished product release specification (**REC 4**).

With the exception of the acceptance criteria for genomic titre and endotoxin, the FP specifications are identical to the FP specifications of Zolgensma. The applicant has provided an overview of the Zolgensma batches that were used to establish the acceptance criteria and OAV101B batch data to demonstrate that the FP conforms to the proposed specifications.

The provided justification is sufficient; the applicant committed to a re-evaluation of the total protein acceptance criterion when additional OAV101B batches are available (**REC 5**). The acceptance criterion for genomic titer was set based on the mean ± 3 standard deviations of batch release and stability data from FP batches. This is acceptable. The endotoxin limit was calculated to meet the compendial requirement of 0.2 EU/kg/hr, using a potential patient weight of 5.81 kg based on the WHO Growth Charts for girls aged 6 months at the 3rd percentile. This is acceptable.

The Applicant intended to only perform partial re-testing of a few selected release parameters upon importation in the EU due to the limited amount of batch material available. The Applicant's justification was not considered sufficient considering that this exceptional exemption is primarily foreseen for imported patient-specific ATMPs and full re-testing in the EU would only require additional 18 out of 197 vials (Major Objection). This issue was solved with the commitment provided by the applicant to transfer the FP release tests that are currently performed in the USA to its testing facilities in EU. (**REC 6**).

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed (as requested) considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Analytical procedures and reference standards

The description of the analytical procedures includes information on method principle, equipment and materials, preparation of standards and controls, method procedure, system suitability criteria, and sample acceptance criteria. The provided information is sufficient.

The results of the method validations do not give rise to questions.

A two-tiered in-house reference standard system is in place. The reference standards, stability test plan, and qualification protocol for future reference standards are the same as for the applicant's registered product Zolgensma, which contains the same active substance. This is acceptable. Sufficient information on the reference standards is included in the CTD.

Batch analysis

Batch analysis data encompassing the clinical batches, development batches, PPQ batches and commercial batches are provided. Overall, the data are consistent and indicate the process is under control.

Container closure

The finished product is presented in 5 mL cyclo-olefin polymer vials. The container material, dimensions and specifications, and information on extractables/leachables are provided. The vial and stopper comply with Ph. Eur.

Sterilisation of the vials is done by irradiation at 12.0-22.0 kGy. As this is different than the reference conditions stated in the Ph. Eur. the validation of the sterilisation process should be provided. The committed to include this information in the dossier (**REC 7**). The stoppers are sterilised with a steam sterilisation process, in accordance with Ph. Eur.

3.3.4. Stability of the product

The applicant proposes a finished product shelf life of 24 months at ≤ -60 °C. This claim is supported by the provided stability data for Process 2, which include 36 and 24 months data for clinical, 9 months data for the PPQ batches and 3 months data for commercial batches. In addition, comparability with the stability of Process 1.0 batches was demonstrated for which 36 months, 24 months and 18 months data are available for batches with the same vector concentration, fill volume and container. Stability is tested at 0, 3, 6, 9, 12, 18, 24, and 36 months, which is in line with the ICH guideline. The tests performed include appearance, pH, osmolality, genomic titre, purity/impurities, potency, capsid distribution, sub-visible particles, and container closure integrity.

As described in the SmPC "Once the dose is drawn into the syringe, it may be held at 2°C to 8°C for up to 24 hours, including a 5-hour maximum time out-of-refrigeration allowance within the 24-hour period. The vector-containing syringe should be discarded if not used within this time period."

In-use stability was evaluated in compatibility studies (compatibility with delivery device at room temperature demonstrated for 24 hours) and in an end of shelf-life in-use study. In the latter study batches at the end of shelf life (currently n=1 for Process 2.0; n=5 for process 1.0) were stored for 14 days at 2-8°C and tested using the test panel as described above. The results support the proposed storage of up to 14 days at 2-8°C at the clinical site.

The applicant committed to complete the ongoing stability studies.

3.3.5. Post approval change management protocol(s)

n/a

3.3.6. Adventitious agents

The risk of adventitious virus and TSE/BSE contamination and the measures taken to address these risks are sufficiently discussed. Virus and TSE safety of materials of human and animal origin used in the manufacturing process is sufficiently ensured. Tests performed on the cell banks, raw material, and in-process are in line with expectations.

Virus validation studies have been performed for the Tween 20 incubation step, the CEX chromatography step and the low pH incubation step. Effective clearance of the enveloped viruses xenotropic murine leukemia virus (XMuLV) and pseudorabies virus (PRV) was demonstrated. No effective clearance of the non-enveloped viruses Hepatitis A Virus (HAV) and minute virus of mice (MVM) was demonstrated, which is not unexpected as the active substance is also a non-enveloped virus. Considering the virus tests performed on the cell banks and harvest, the limited capacity of the manufacturing process to remove non-enveloped viruses can be accepted for this product. The applicant committed to include a test for

MVM in the qualification protocol for future WCBs (**REC 8**).

Genetically modified organisms (GMO)

OAV101 is considered a genetically-modified organism and as such a consultation with the GMO authorities was performed. For more information about the environmental risk assessment of the GMO please refer to section 5.1.5.

3.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

During assessment only one Major Objection was raised on the batch release upon importation to the EU. The applicant provided justification for the requested waiver and with the commitment to transfer testing to the EU the issue was resolved.
A few recommendations remain that do not affect the benefit-risk profile of this product.

3.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

The MAA for Itvisma is considered approvable from a quality point of view.

3.6. Recommendations for future quality development

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

REC 1 – The applicant commits to including the qualitative composition of the commercially derived culture media in Module 3.2.S.2.3

REC 2 - The applicant commits to introducing pressure monitoring for sterile filtration

REC 3 - The applicant commits to updating aseptic process simulation (APS) approach to include the demonstration of representative containers and worst-case processes are captured every 6 months

REC 4 - The applicant commits to the development, validation, and implementation of an independent, direct capsid titer determination method suitable for Finished Product release specification.

REC 5 - The applicant commits to re-analysing the total protein acceptance criterion for finished product release once 30 OAV101B batches have been manufactured and the corresponding analytical data are available.

REC 6 - The applicant commits to transferring the remaining finished product release methods to their EU testing facilities.

REC 7 - The applicant commits to including in the dossier, the validation data demonstrating that the finished product vial sterilization process achieves the required sterility assurance level (SAL).

REC 8 -The applicant commits to revising the WCB qualification protocol for future cell banks to include a test for MVM.

4. Non-clinical aspects

4.1. Introduction

One single dose toxicity study was conducted in line with the GLP principles (i.e. study no. 1970356). This study was conducted in 2020/2021 by Covance Laboratories Inc (Madison, Wisconsin). At the time of study conduct, this site was in compliance.

4.2. Pharmacology

4.2.1. Pharmacodynamics

4.2.1.1. Primary pharmacodynamics

All the submitted studies / publications have already been evaluated during marketing authorisation application for Zolgensma and are addressed below.

In vivo proof of concept for ICV/IT dosing

In the publication from Meyer et al. 2015, the relation between OAV101 dose and efficacy upon ICV injection was investigated in the mouse model for SMA. SMNΔ7 mice receiving ICV the highest dose of AVXS-101 (3.3×10^{13} vg/kg) 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 (2.7×10^{12} vg/kg and 1.0×10^{13} vg/kg) 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. In SMNΔ7 mice administered doses $\geq 1.8 \times 10^{13}$ vg/kg, righting reflex (motor performance), assessed at P15, was almost completely restored to wild-type levels and locomotor activities (rearing and ambulatory movements) assessed at P30 were similar to wild-type mice. The applicant could further demonstrate that a dose level of 1.8×10^{13} vg/kg scAAV9.CBA.GFP, a surrogate vector expressing GFP instead of SMN, resulted in 20-40% of transduced spinal motor neurons, when administered ICV in neonatal mice (Meyer et al., 2015).

Overall, 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. Considering that PND1 pups weigh approximately 1.5 gram; this would translate to a dose of $4.8 \times 10e^{10}$ vg. As CSF volume is approximately 35 µl in these mice and equals ~ 120 ml in patients (factor difference of 3429), the dose to mice would correlate to a clinical dose of 1.6×10^{15} vg. A clinical dose of ~ 2.3×10^{14} vg was tested in the phase I clinical study, which was however not continued, because of DRG toxicity. Regarding the dose in Yorkshire pigs, considering a weight of 1.35 kg at PND5 and CSF volume of 100 ml, the dose would correlate to 1.3×10^{13} vg as clinical effective dose, which would be feasible in patients. However, as these studies were conducted in PND1 mice and PND5 pig, a direct translation of the efficacious dose in animals towards an efficacious clinical dose. Thus, a rough comparison, not taking into consideration ICV versus IT and differences between mice and men, would conclude that the clinically targeted intrathecal dose is not achievable in patients. Support for the clinical dose should be derived from clinical data.

Additional support for treatment rationale in Piglets

In the study from Duque et al. 2015, 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, such as hind-limb weakness. These shSMN1 treated piglets were transduced with AVXS-101 (AAV9 vector carrying the SMN1 transgene) either presymptomatic (PND6, dose 8 x 10^{12} vg/kg) or symptomatic (PND 33-36, dose 2-3.8 x 10^{13} vg/kg).

Immunostaining of piglet spinal cord reveals a strong tropism of AAV9 for the motor neurons, with glial cells transduced only on rare occasions. Upon presymptomatic treatment on PND6, SMA disease phenotype (such as hind-limb weakness) was completely prevented. Electrophysiology biomarkers including electromyography (EMG), compound muscle action potential (CMAP) and motor unit number estimation (MUNE) were used to assess the impact of OAV101 treatment on the function of the motor unit. At P54, fibrillation potentials were observed via EMG in muscles from scAAV9.shSMN-injected piglets, while these were not present in the control animals. In addition, significant decreases in CMAP and MUNE were observed in scAAV9.shSMN-injected animals compared to controls. Upon symptomatic treatment at PND33-36, partial amelioration of the disease was observed. While hind limb weakness remained in 3 out of 5 treated animals, the severity of the weakness was reduced. In the other 2 animals revealed progression of hindlimb weakness toward severe paresis, however, complete paralysis has not been observed, while untreated animals were unable to stand at PND38 - PND55 (Duque et al., 2015).Although symptomatically treated piglets still responded and benefitted from treatment with AVXS-101, these experiments suggest that early delivery of the therapeutic vector is critical for optimal efficacy.

Evaluating the biodistribution in mice, piglets and monkeys (macaque fascicularis)

For evaluation of transduction in the brain and SC following IT vector administration, a GFP-expressing surrogate vector (scAAV9.CBA.GFP) that is similar to OAV101 with the same AAV9 capsid and CBA promoter but expressing GFP instead of SMN has been used.

In the study from Foust et al. 2009, distribution of AAV9 and subsequent transgene expression was assessed in neonatal and adult mice. Targeting of AAV9.CB.GFP, a AAV9 vector similar to AVXS-101 but containing a sequence for GFP protein, was studied in wild-type C57BL/6 mice that received an IV injection of 4 × 10^{11} vg/mouse (3.3 × 10^{14} vg/kg) on PND1 or 2, when the blood-brain barrier in mice is not yet closed. GFP expression at 10 and 21 days post-injection was identified in heart and skeletal muscle and spinal cord, primarily in dorsal root ganglia (DRG) and lower motor neurons. Widespread GFP expression was found in the brain, which also persisted up to 21 days. Co-labelling for ChAT and GFP expression within the spinal cord revealed many ChAT-positive cells expressing GFP throughout all cervical and lumbar sections examined, indicating widespread motor neuron transduction. At 10 days post-injection, 56 ± 1.9% of ChAT-positive motor neurons strongly expressed GFP in lumbar spinal cord sections and 60 ± 2.9% at 21 days post injection (n=4-5 animals per time point). 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. This study supported IV treatment at early age, which might not be so relevant for the current application using IT administration.

Biodistribution in mice was also studied by ICV administration of AAV9.CB.GFP construct, which appeared to have similar biodistribution as AVXS-101. With the highest dose of the AAV9.CB.GFP vector, 46, 47

and 72% of cervical, thoracic and lumbar motor neurons were transduced respectively. A comparison of the mRNA transcript expression levels for the *SMN* or GFP gene products in OAV101- or scAAV9.CB.GFP-treated mice, respectively, demonstrated dose-dependent increases in transgene expression in the cervical, thoracic, and lumbar spinal cord. As dose response of the mRNA levels was similar between OAV101 and scAAV9.CBA.GFP, as such GFP was regarded indicative for SMN gene expression patterns when same promoter is used. This approach can be agreed.

In a study from Bevan et al. 2011, distribution of the scAAV9.CB.GFP vector and expression of its transgene was investigated in two groups of three 5-day old piglets that were dosed either via the intrathecal (at L5) or via the intracisternal (at the base of the skull) route with 5.2 x 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 DRG and spinal cord gray and white matter. scAAV9.CB.GFP injection into either the cisternal space or the intrathecal space resulted in extensive motor neuron and large ventral horn neuron transduction at all levels of the spinal cord. In the brains, highest expression levels were seen in cerebellar Purkinje cells, medulla nerve fibres 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.

Biodistribution was also studied in (juvenile) one year old macaque fascicularis (2 kg), which 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 (Meyer et al. 2015). Maximal transduction efficiency was achieved when animals were held in the Trendelenburg position for 10 minutes post-injection. GFP expression was 55 ± 2.0%, 62.3 ± 2.4% and 79.6 ± 1.2% (n=3) for cervical, thoracic and lumbar regions, respectively. In mice, an ICV dose of 3.3 x 10^{14} 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 macaque as compared to mice, however the age of the animals was not considered in such a comparison.

Efficient transduction and partial amelioration of the SMA disease phenotype in the post-symptomatic setting was observed despite using the same AAV serotype for both induction of SMA phenotype at PND5 (scAAV9.shSMN) and expression of human SMN by administration of OAV101 at PND33-36. This observation is in line with published reports showing that pre-existing anti-AAV9 neutralizing antibodies have a minimal impact on transduction of AAV9 vectors administered IT in brain and spinal cord of NHPs (Gray et al., Gene Ther 2013;20:450-59; Guibinga et al. Mol. Ther. Methods Clin. Dev. 2024;32:101371).

4.2.1.2. Secondary pharmacodynamics

For Itvisma, EMEA/H/C/004750/0000, a study was submitted to show the effect of IV OAV101 treatment on a peripheral organ, the heart, actually reversing disease related symptoms. Upon request, the applicant submitted this study, from Bevan et al., (2010), in which it was shown that in the severe SMA mice model (SMNΔ7) early treatment (PND1) resulted in improved left ventricle (LV) remodelling and fully corrected heart rate. The extent of dilation as well as wall thinning were attenuated and returned toward WT values in treated SMNΔ7 mice.

However, treatment with OVA101 for the current indicated patient population might not result in such severe phenotype as was observed in the SMNΔ7 mice model, especially with regard to the peripheral symptoms. Therefore, the secondary effect regarding delaying peripheral damage, or even improving peripheral symptoms of the disease, might not be translatable to the indicated patient population and

the route of administration currently applied for. If occurring at all, support for improvement of peripheral symptoms related to the disease, should be collected from clinical data.

4.2.1.3. Safety pharmacology

No studies were submitted.

4.2.1.4. Pharmacodynamic drug interactions

No studies were submitted.

4.2.2. Pharmacokinetics

4.2.2.1. Absorption

No absorption studies have been conducted with OAV101.

4.2.2.2. Distribution

In neonatal mice single ICV administration of $3.3x10^{13}$ vg/kg OAV101 on PND1 resulted in transgene mRNA expression in all analysed regions of the spinal cord 24 weeks post-dosing. Using the same dose (ICV) or a dose of $1.1x10^{14}$ vg/kg (IV) administered at PND1, vector distribution was found in testis and ovary, but not in germline cells, 3 and 8 weeks post-dosing. Distribution to gonads was lower compared to liver transduction (study no. 2170026). Administration of $1.1x10^{13}$ or $1.1x10^{14}$ vg/kg (IV) OAV101 in adult male mice did also result in biodistribution of the vector to testes, but vector was not found in maternal (undosed females) or foetal tissue after mating with OAV101-dosed males (study no. 2470261).

In young cynomolgus monkeys, dosing of $2x10^{13}$ vg/kg resulted in SMN mRNA expression in CNS and peripheral tissues 12 months post-dosing (study no. CSF-AAV9-SMN-NHP 001). In the pivotal NHP toxicity study, $1.2x10^{13}$, $3x10^{13}$ or $6x10^{13}$ vg/animal biodistribution of vector and transgene were analysed in various tissues at 6 weeks and 12 months post-dosing of juvenile monkeys (study no. 1970356). Vector distribution was widely observed both in the CNS and periphery, especially in the liver and DRGs, with concentrations in (at least) spinal cord and DRGs persisting over time. Transgene mRNA expression was expressed in all examined tissues, especially in heart, liver, muscles and testes (ovaries not analysed). Persistence of transgene expression over time could not be determined due to lack of certain (CNS) tissue samples and considerable interindividual variability. Use of contrast agents (study no. RPT-1591) or immune-suppressants (study no. 1970589) did not significantly affect the biodistribution in juvenile monkeys. In addition, in peripubertal monkeys, pre-existing anti-AAV9 antibodies in serum did not impact the biodistribution in CNS, although a reduction in distribution was observed in DRGs and several peripheral tissues.

To support their choice for the IT route of administration, the Applicant further provided a comparison of vector distribution and transgene expression between IT ($1.2x10^{13}$ vg/animal, study no. 1970356) and IV ($1.1x10^{14}$ vg/kg, study no. 2070093) administration of OAV101 in NHP, 6 weeks post-dosing. In both studies, male and female monkeys around the same age (13-19 months for IT and 14-17 months for IV) and the same animal numbers per sex per group were used. The type of histopathological findings and tissues involved in biodistribution were comparable between IV and IT administration. Generally, IV administration resulted in higher vector DNA and SMN mRNA concentrations in peripheral tissues. This was accompanied by more pronounced systemic toxicity, although this is usually manageable in patients

with temporary use of corticosteroids (see Zolgensma SmPC). In contrast, IT administration resulted in more extensive CNS toxicity, i.e. the number of tissues involved was larger. Severity of DRG and spinal cord/nerves toxicity appeared to be more or less the same for both administration routes. Peripheral nervous system (PNS) degenerative signals were mostly observed following IT administration. This was related to the higher vector genome levels in the neuronal tissues, as expected with this local route of administration. Despite these higher vector transduction levels, IT administration did not appear to result in higher mRNA levels in CNS and DRGs. In addition, there was a trend towards lower vector (and transgene) concentrations over time with both administration routes.

4.2.2.3. Metabolism

No metabolism studies have been conducted with OAV101.

4.2.2.4. Excretion

No excretion studies have been conducted with OAV101.

4.2.2.5. Pharmacokinetic drug interactions

No PK drug interaction studies have been conducted with OAV101.

4.2.2.6. Other pharmacokinetic studies

No other PK studies have been conducted with OAV101.

4.3. Toxicology

4.3.1. Single-dose toxicity

Toxicity in mice

ICV administration of $3.3x10^{13}$ vg/kg OAV101 to neonatal wild-type mice did not result in any signs of toxicity until week 24 post-dosing (study no. CSF-AAV9-SMN-MOUSE-001). Nevertheless, this route of administration induced anti-AAV9 capsid antibodies in serum. In a comparable study, no signs of toxicity were observed until week 12 post-dosing (study no. CSF-AAV9-SMN-MOUSE-002). Immunogenicity was not evaluated in this study. Both murine toxicity studies were non-GLP.

Toxicity in NHP (non-GLP)

Young male NHP (4-6 months old, study no. CSF-AAV9-SMN-NHP-001) received a single IT administration of $2x10^{13}$ vg/kg and were followed for 18 months. Except for a transient increase in platelets (3/5 treated animals) during the first 6 months, no significant OAV101-related toxicity was observed in these animals. The treatment induced anti-AAV9 capsid antibody and anti-human SMN antibody titres. There was no apparent T cell response against the vector capsid or transgene protein.

In two other toxicity studies (study no. 1970589 and RPT-1591), the Applicant determined the potential impact of immune-suppressants or contrast agents on the distribution and neuronal toxicity of $3x10^{13}$ vg OAV101/animal IT administration in juvenile NHP. The use of systemic prednisolone or rituximab + everolimus before OAV101 dosing and in the weeks post-dosing did not result in clear amelioration/prevention of inflammatory and neuronal biomarkers and microscopic findings nor in

pronounced changes in biodistribution/persistence or immunogenicity. Lack of beneficial effects of immunosuppressants (mycophenolate mofetil and rapamycin) on DRG toxicity has also been observed by others (Hordeaux *et al*., Mol Ther Methods Clin Dev, 2018). Transduction efficiency of the spinal cord and DRG toxicity of the vector was not impacted by the concurrent use of iohexol-based contrast agents.

Toxicity in NHP (GLP)

The Applicant conducted one GLP toxicity study in NHP (study no. 1970356), which is considered the pivotal safety study for this non-clinical dossier. Juvenile animals (13-19 months old) were once IT injected in the lumbar region with $1.2x10^{13}$, $3x10^{13}$ or $6x10^{13}$ vg OAV101 or with a vehicle control. All animals also received an injection with 0.2 mL contrast agent Omnipaque™ 180/animal prior to administration of OAV101/vehicle control. After administration, animals were followed for 6 weeks (interim analysis) or 12 months (final analysis).

IT administration of OAV101 did not result in (OAV101-related) body weight changes or clinical observations, including those related to specific organ systems (e.g. respiratory, neurologic or cardiac functionality), during the whole evaluation period.

At the interim analysis, increased aminotransferase levels in serum were observed in treated compared to control animals at at $\geq 1.2x10^{13}$ vg, which was also observed in non-clinical studies that were conducted with IV administration of OAV101 (Zolgensma). Increased aminotransferase levels were related to single cell necrosis of hepatocytes in animals treated IT with $\geq 3x10^{13}$ vg OAV101/animal (at interim analysis only). Considering the high vector transduction level of the liver, these effects appear to be inherent to OAV101 treatment. This indicates that even after IT administration a substantial part of the product enters the systemic circulation. OAV101-related findings in clinical pathology during the first 6 weeks post-injection normalised thereafter, indicating resolution/reversibility of these markers and the corresponding histopathological findings.

Dose-dependent microscopic abnormalities of inflammation and degeneration were found during the interim analysis in the central and peripheral nervous system: in the brain stem, dorsal root ganglia, trigeminal ganglia, spinal cord, dorsal spinal nerves/nerve roots, and in peripheral nerves (such as sciatic nerve). This was accompanied by increased levels of NfL, a biomarker for axonal damage, in CSF and serum. Microscopical findings in (at least) the DRG and spinal cord were accompanied by very high vector transcript levels, suggesting that high levels of vector transduction and/or transgene expression have resulted in neuronal pathology. Moreover, axonal degeneration in brain and spinal cord are likely secondary to the neuronal degeneration in the DRGs. At terminal necropsy, there was no increased severity of the axonal degeneration observed in the brain stem, indicating non-progression. In addition, histopathology findings in the cerebrum/cerebellum, in the spinal cord, in the DRGs and trigeminal ganglion and at the injection site were observed with decreased incidence and severity compared to the interim analysis at week 6. At the lowest dose, findings were even no longer observed, except for those in the DRGs. Evidence of scarring (satellitosis/gliosis) indicated partial/trend to resolution and tissue remodelling after neuronal loss, although this was not accompanied by clinical (neurological) observations in the monkeys. Pathological findings observed in peripheral nerves during interim analysis were no longer found at terminal necropsy, likely as a consequence of resolution/reversibility of the degeneration and inflammation.

Single treatment with OAV101 resulted in an antibody response against the vector in serum and CSF in most animals, which persisted during the whole study period.

4.3.2. Repeat-dose toxicity

Because the product is intended for single dose injection, no repeat-dose toxicity studies have been

performed.

4.3.3. Genotoxicity

As OAV101 is a non-integrative vector, that theoretically only integrates into DNA to a very low extent, the mutagenic potential is regarded very limited.

4.3.4. Carcinogenicity

As OAV101 is a non-integrative vector, that theoretically only integrates into DNA to a very low extent, the mutagenic and the tumorigenic potential is regarded very limited. Data from two clinical cases of a tumour in a Zolgensma treated patient, indicate that integration events were infrequent and randomly distributed rather than resulting into a dominant clone. In these two cases, a role for a vector integration event in tumor progression and a causal relationship with OAV101 was not established. However, recent data emerging from the clinical program of another investigational gene therapy, prompted a reevaluation of the reflection of the tumorigenic risk of Itvizma in the SmPC, which is currently reflected as *'theoretical risk of tumourigenicity due to potential integration of AAV vector DNA into the genome'* (see section 6.4.4).

4.3.5. Developmental and reproductive toxicity

Fertility and Early Embryo Development

The Applicant submitted 2 FEED studies. In study no. 2470261, 12 weeks old male FVB/NCrl mice were dosed IV with vehicle, 1.1x1013 or 1.1x1014 vg/kg OAV101 and with 16 weeks allowed to mate with undosed females. Mating was allowed for 7 consecutive days. Whereas in the other study (no. 2470188), 10 weeks old female FVB/NCrl mice were dosed IV with vehicle, 1.1x1013 or 1.1x1014 vg/kg OAV101 and allowed to mate 14 days later with undosed males. Mating was allowed for 14 consecutive days. The choice for the period between dosing and mating as well as the period in which mating was allowed was not further justified.

In both studies there were no treatment-related findings with regard to body weight (change), food consumption, clinical symptoms or macroscopic evaluation. Furthermore, reproductive performance of the dosed males and females did not deviate from the control animals. This indicates that treatment with OAV101 might not have an impact on the fertility and early embryonic development in animals, which might be reassuring for the reproductive safety of OAV101 in human as well.

Only one effect was observed in treated animals, namely the oestrous cycle of dosed females tended to be longer, which might be treatment-related, but this did not translate to deviating findings with regard to reproductive performance.

Biodistribution was only analysed in dosed F0 males, undosed females and their progeny. However, in study 2470188, biodistribution was not analysed. The Applicant notes that Following completion of GD 13 uterine exams, there was an insufficient number of females with known mating dates to perform tissue collection for biodistribution analyses. Thus, foetal biodistribution could not be assessed in this study. Besides absence of foetal biodistribution data, also biodistribution data in dosed females is lacking therefore it is not confirmed that ovaries were indeed positive for the vector.

Furthermore, the biodistribution towards testes of 5/5 dosed males in study 2470261 was compared to distribution towards the testes to 3/6 males in study 2170026, that was submitted as part of the LEG procedure for Zolgensma (EMEA/H/C/004750/LEG024.1). The vector genome levels found in testes in

study 2470261 were approximately 100 times higher as compared to the levels found in testes in study 2170026. The animals in these later studies were dosed on PND 1, and vector levels were analysed 8 and 12 weeks later, whereas animals in study 2470261 were dosed at 12 weeks of age and likely analysed 6 weeks later.

With regard to the mating period, the applicant explained that a period of two weeks was used as per the ICH S5(R3) guideline. With regard to the dosing before mating, it was chosen to dose females two weeks before mating, as ovarian follicles needs two weeks to mature. However, male mice were dosed four weeks prior to mating as the process of sperm production takes approximately 34 to 35 days to complete in a mouse. A single sperm cell takes roughly 35 days to develop from a stem cell into a mature spermatozoon. In both cases, it was the intention to affect gametes at every stage of development, as well as the gonads to be able to notify OAV101's potential for fertility and early embryonic adverse effects. However, the data from the FEED studies do not indicate the likelihood of OAV101 transducing gametes during the chosen dosing and mating periods.

The presence of the vector was not analysed in tissue from just a few foetuses and justify the absence of biodistribution data to ovaries in the dosed females in study 2470188 (female FEED study). The applicant explained that the females for whom mating was confirmed were assigned towards the fertility part of the study. The females for whom mating could not be confirmed, had to be euthanized by GD13, and consequently there was no option to collect tissue on GD18 for foetal biodistribution analysis. It is endorsed that the fertility endpoints were acquired in this study, and OAV101 distribution to ovary tissue is shown in other studies.

In the male FEED study, 12 weeks old female FVB/NCrl mice were administered vehicle control article 1.1 x 1014 vg/kg OAV101 via IV bolus injection once, resulting in 0.979 copies per diploid genome at 18 weeks. In the germline study, the males were dosed 1.1 x 1014 vg/kg OVA101 on PND1 and necropsied at age 8 weeks or 24 weeks. The mean OAV101 DNA concentrations in the testes were 0.0786 copies/dg at 8 weeks and 0.0037 copies/dg at 24 weeks. Although levels were both very low, minimal tissue growth is expected to occur as OAV101 is given to adult mice, that might result in higher vector levels in adult-dosed males due to less dilution from tissue growth compared to neonatal dosing.

Embryonic and Foetal development

The applicant conducted an EFD study to investigate potential embryonic and foetal effects of treatment with OAV101. Time-mated female FVB/NCrl mice were IV dosed $1.1x10^{13}$ or $1.1x10^{14}$ vg/kg OAV101 on GD 6. On GD18 these animals underwent caesarean section and pregnancy status and uterine contents were recorded. Upon caesarean section no OAV101-related effects on pregnancy status and uterine contents, gravid uterine weight, corrected maternal body weight/body weight gain, covariate adjusted foetal body weight, or foetal external, visceral, or skeletal examinations were noted. The assay used for determination of OAV101 material in maternal or foetal tissues was considered fit for purpose. OAV101 DNA was found in maternal tissue, among which ovaries, placenta and uterus. Foetal tissue did not test positive for OAV101 DNA (see Table 4 below). Concentrations increased dose-dependently.

Table 4. Presence of OAV101 in maternal and foetal tissue in copies per diploid genome

Dose Level	0	1.1×10^{13} vg/kg	1.1×10^{14} vg/kg
Maternal Liver	BLQ	3.3156	80.2796
Ovaries with Oviducts	BLQ	0.5615	4.8916
Placenta	BLQ	0.9192	9.1413
Uterus	BLQ	0.1518	2.2833
Fetal Liver	BLQ	BLQ	BLQ
Caudal fetus	BLQ	BLQ	BLQ

BLQ = Below the level of quantification

The NOAEL for maternal and developmental toxicity is 1.1x1014 vg/kg, and it is accepted based on the current data, IT treatment with OAV101 presents a negligible risk for embryonic and foetal toxicity in the intended patient population.

Absence of studies addressing pre and postnatal development and juvenile toxicity is accepted.

Overexpression of SMN1 in Oocytes

The consequences of overexpression of SMN1 (due to OAV101 transduction) in gonads or gametes (oocytes in particular) on fertility, conception and early embryogenesis were discussed. It was explained that SMN is an RNA binding protein, resulting in a lot of interaction amongst other with proteins that regulate essential cellular functions such as DNA replication and repair, transcription, pre-mRNA splicing, translation, stress granule formation, macromolecular trafficking, signal transduction, and cytoskeletal dynamics. Therefore, overexpression of SMN1 might compromise fertility, conception, and early embryogenesis. However, no transduction of germline cells and no negative impact on fertility, mating, or embryofetal outcome at (or above) clinically relevant doses for both IV and IT administration routes have been shown for OVA101.

Since both routes deliver ≤1 copy of OAV101 per cell, the risk of SMN overexpression is low or improbable. This is confirmed by levels of vector DNA found in gonads, which stay below 1 and do not suggest high levels of SMN1 expression per cell. SMN protein itself is naturally present in oocytes and testes, being essential for embryonic development, and slight to moderate overexpression is not suggested to be harmful.

Furthermore, Sabatino et al. (2022) concluded that the risk of insertional mutagenesis and sustained overexpression of genes delivered by AAVs remains minimal under clinically relevant dosing regimens.

As there are no adverse reproductive or developmental outcomes observed in animal models in combination with a very limited risk of overexpression of SMN1 gene it can be concluded that treatment with OAV101 will not place a patient at risk regarding fertility, conception, or early embryogenesis.

It is reported in a literature study (Zhao et al, 2020) that also in adult mice upon IV AAV9 treatment, an ISH signal was absent from oocytes or from the seminiferous tubules. Similar result was obtained with OAV101 dosing at PND1 in study 2170026, which suggest that age might not be a factor to consider in mice. There is no literature data in non-human primates using molecular localization approaches to look for the presence of viral genome and/or transgene product in the gonadal cells, which makes it difficult to place the data from the Applicant in perspective regarding the influence of the age and sexual maturity at time of administration, and evaluation timepoint post-administration, etc. In a literature review with regard to germline transmission data for AAV gene therapies suggest there is little risk for a recombinant AAV (rAAV, such as onasemnogene abeparvovec) to modify germline DNA and be passed on to the following generation (Jakob et al 2005, Schuettrumpf et al 2006, Pañeda et al 2009, Ferla et al 2017, Watanabe et al 2017, Watanabe et al 2018, Kanatsu-Shinohara et al 2022). This is endorsed. OAV101 uses a recombinant AAV9 vector with all wild-type DNA removed from the capsids (including the key genes (i.e., Rep ORF involved in integration events), except for the Inverted Terminal Repeats (ITRs), which reduces the risk of integration compared to wild-type/natural AAV, also because the recombined virus is designed to remain episomal in the cell nucleus. This further reduces the low risk of integration by these non-replicating rAAV vectors.

4.3.6. Toxicokinetics and exposure margins

Toxicokinetic studies have not been performed for Itvisma, which is in line with the product type.

4.3.7. Local tolerance

No dedicated local tolerance studies have been performed.

4.3.8. Other toxicity studies

Study 2170026: OAV101: Single dose intravenous or intracerebroventricular germline transduction and integration study in neonatal FVB/NCrl mice with up to a 24-week evaluation period

The Applicant conducted a germline transduction and integration study. OAV101 as a single IV or ICV injection at doses of 1.1 x 10^{14} vg/kg or 3.3 x 10^{13} vg/kg, respectively, was administered to neonatal FVB/NCrl mice on PND1. It was not discussed whether the age of the animals at dosing would be of influence for the likeliness of the vector transducing oocytes and the risk for integration (which is considered highest at maximum vector levels). It is not known whether oocytes would get more accessible when maturing at reproductive age as compared to oocytes present in mice that are not yet sexually mature.

OAV101-related reductions in mean body weight and mean body weight gain were noted for animals administered 1.1 x 10^{14} vg/kg IV (both sexes), which were regarded non-adverse. Body weight changes were not observed upon ICV administration of 3.3 x 10^{13} vg/kg OAV101.

The presence of OAV101 DNA in gonads was examined 3-(female), 8-(male) and 24-(both) weeks post-dose by means of detection either via digital droplet Polymerase Chain Reaction (ddPCR) in the tissues (including gonads and sperm) or via in situ hybridization (ISH). Gonad examination included interstitial cells, seminiferous tubules, ovaries and oocytes.

ddPCR

OAV101 was detected by ddPCR at highest levels in liver of all animals, both sexes via both routes of administration (RoA) at all timepoints, apart from one ICV administered animal. Upon IV administration OAV101 DNA was detected in the ovary 3- and 24-weeks post-dose (3/6 animals), as well as in testis 8- and 24-weeks post-dose (of 2/6 animals), but OAV101 was not detected in sperm. Upon ICV administration OAV101 DNA was detected in the ovary at 3-weeks post-dose (1/6 animals) and at 24-weeks post-dose (3/6 animals), but OAV101 was not detected in the testis or sperm at any timepoint. Apparently, IV dosing results in higher level of OAV101 positive ovaries and testes, as compared to ICV dosing. This may indicate that upon IV dosing higher levels of OAV101 will be present in the systemic circulation to transduce ovary or testis tissue. As males were not analysed at 3 weeks and females not at 8 weeks post-dose, the difference in presence of OAV101 between females and males at timepoints of 3- and 8-weeks post-dose has no specific meaning.

ISH

Hybridization with the OAV101 AS and S probe produced no signal in the testes or ovaries from the select vehicle control tissue analysed but did detect signal in the liver sections from all of the IV and ICV dosed male and female animals tested. With the AS probe this signal was cytoplasmic and nuclear, consistent with detection of vector mRNA and DNA, while with the S probe the signal appeared most often as punctate positivity consistent with detection primarily of vector DNA.

Detection OAV101 by ISH revealed in males a signal in testes 8-weeks post-dosing. This rare vector signal was observed in interstitial cells, but signal was absent in seminiferous tubules. In females, signal was detected in ovaries 3-weeks post-dosing. This rare to minimal vector signal was observed in granulosa cells and cortical stromal cells, but signal was absent from oocytes.

Study 2220183: AAV vector expression in cynomolgus macaque gonadal tissue of scAAV9-CB-GFP and scAAV9-CB-mCherry 28 days after administration

A molecular localization study on gonadal tissues was undertaken to evaluate and characterize the transgene expression (GFP or mCherry) using immunohistochemistry for the reporter transgene products green fluorescent protein (GFP) in ovary and GFP or mCherry in testes from cynomolgus macaques, which were sacrificed 28 days post-dosing. The scope of the study, designed to distinguish transduction in somatic versus germ cells in gonadal tissue, was limited to investigative/exploratory activities on the female gonadal tissues (ovaries) from the animals in Study 2070139, and male gonadal tissues (testes) from the animals in Study 2170445 and Study 2170367.

Females

GFP immunohistochemistry was performed on ovaries from female animals aged approximately 13-17 months (body weight 1.2 to 1.8 kg) at study initiation dosed with scAAV9-CB-GFP by the IT and ICM route in Study 2070139. No staining was observed in the ovaries of vehicle control animals or with an irrelevant rabbit Ig control antibody. The transgene protein (GFP) was detected in a subset of oocytes and ovarian stromal cells by either route at 1.0×10^{13} or 3.0×10^{13} vg/animal and indicates transduction of female germ cells with this vector under the experimental conditions. It should be noted that these juvenile female cynomolgus monkeys would normally be regarded sexually immature. According to the applicant, the animals were already cycling, determined by evidence of Graafian follicles in ovaries. It is not explained by the Applicant how it was possible that these young animals were already cycling.

Males

GFP or mCherry immunohistochemistry was performed on testes from sexually mature male animals dosed with 3.0×10^{13} vg/animal scAAV9-CB-GFP by the IT route in Study 2170367 or with 7.0×10^{13} vg/kg scAAV9-CB-mCherry by the IV route in Study 2170445, respectively. Rare to minimal GFP immunohistochemistry staining was observed in cells of the testicular interstitium in 4 of 12 animals following IT administration Study 2170367. Mild to moderate mCherry staining of testicular interstitial cells was observed in 3 of 3 animals dosed with scAAV9-CB-mCherry following IV administration. No mCherry or GFP immunohistochemical staining was observed in the seminiferous tubules (including germ cells) of animals in Study 2170445 or Study 2170367, respectively.

4.3.9. Ecotoxicity/environmental risk assessment

The ERA contains an overview of the AAV viral vector. In other modules of the MAA-dossier information on the manufacturing plasmids is present, vector maps of all plasmids are provided accompanied by a description of the components on the transferplasmid and helperplasmids. Also, information on the validated replication competent AAV (rcAAV) assay was presented in another part of the dossier.

The applicant states that the plasmid master cell bank (MCB) has been fully characterised. Plasmid identity is confirmed by restriction digest and Sanger sequencing. The molecular characterisation of OAV101B has been performed adequately.

The potential presence of rcAAV in OAV101B as a contaminant, or as an indirect consequence of homologous recombination with wild type AAV and OAV101B in vivo, is not considered to constitute an environmental risk, as this rcAAV is similar to wildtype AAV, which is already globally endemic and a non-pathogenic virus by nature. Moreover, the drug substance is tested as part of manufacturing and no rcAAV was detected.

The applicant refers to published literature that support lower infectivity of shed vector (compared to newly produced vector) and the degradation of AAV9 vector in activated sludge, as found in wastewater

treatment facilities. The applicant claims that shed OAV101B can be practically considered as non-infectious. It is agreed upon that the amount of vector that is shed by patients is transient and peaks in the days after administration after which it declines. Shedding is determined by PCR based methods that detect vector DNA and do not necessarily reflect intact infectious particles. However, based on all information present, the time at which it can be concluded that no infectious particles can be shed by treated patients cannot be determined.

The applicant suggests that no precautions for handling human waste are necessary. Human waste (e.g. feces, urine) could contain excreted vector particles. Shedding will occur for a very limited time after administration and the level to which individuals other than the patient will be exposed in worst case is low, and orders of magnitude lower than the amount of particles administered to the patient. The applicant argues that these particles are less infectious compared to vector particles in the drug product. Yet, the presence of infectious vector particles in excreta such as human waste, although considered low, cannot be fully excluded. However, considering the characteristics of the vector and the transgene, hazardous effects of these particles in the amount that third individuals are exposed to in a worst case scenario are not expected. Further spread of these particles in the environment is considered to be limited. The rationale of the applicant can be followed. The statement that no precautions for handling human waste are warranted for OAV101B is agreed upon.

Several measures have been taken by the applicant to minimize the likelihood of spread in the environment or to non-target individuals, including medical personnel. These are stated in the SmPC and Package Leaflet. The measures include proper transport and waste treatment, the use of personal protective equipment for medical personnel handling or administering the AAV vector, and procedures in case of a spill. Recommendations for treated individuals are given regarding sexual contact, pregnancy and breast feeding, and donation of blood, organs, tissues, or cells for transplantation. These are considered appropriate.

Considering the above, the risk to the environment that is related to the use of OAV101B is considered negligible.

4.4. Overall discussion and conclusions on non-clinical aspects

4.4.1. Discussion

Pharmacology

Primary pharmacology

The Applicant provided studies to support the treatment rationale of intrathecal (IT) administration of OVA101B in two models of the disease, SMNΔ7 mice and shSMN1-treated piglets. Both the SMNΔ7 mouse model, which is a widely used SMA model, and the juvenile piglets, in which SMA pathology is induced by shRNA-mediated knock-down of endogenous SMN expression by administering an scAAV9.shSMN vector at PND5, are acceptable.

Early intracerebroventricular (ICV) treatment in mice and presymptomatic IT treatment in piglets with OAV101 is able to reverse or prevent disease symptoms. Support for later treatment in (less severe) disease models is not provided. The efficacious dose in the mouse model upon ICV administration at PND1 appeared 3.3 x 1013 vg/kg and an efficacious dose in the presymptomatic shSMN1 piglets treated IT at PND6 appeared 8 x 1012 vg/kg. With regard to the efficacious dose in mice, cconsidering that PND1 pups weigh approximately 1.5 gram, it would translate to a dose of 4.8x10e10 vg/animal. As CSF volume is approximately 35 µl in these mice and equals ~ 120 ml in patients (factor difference of 3429), the dose to mice would correlate to a clinical dose of 1.6x1015 vg. A clinical dose of ~

2.3x1014 vg was tested in the phase I clinical study, which was however not continued, because of DRG toxicity. Thus, a rough comparison, not taking into consideration ICV versus IT and differences between mice and men, would conclude that the clinically targeted intrathecal dose is not achievable in patients. Support for the clinical dose should be derived from clinical data. Regarding the dose in Yorkshire pigs, considering a weight of 1.35 kg at PND5 and CSF volume of 100 ml, the dose would correlate to 1.3 x 1013 vg as clinical effective dose, which would be feasible in patients. However, as these studies were conducted in PND1 mice and PND5 pig, a direct translation of the efficacious dose in animals towards an efficacious clinical dose is not possible.

Furthermore, the transduction of target motor neuron cells is supported with evaluation of presence of the vector / transgene expression in mice, piglets and cynomolgus monkey after ICV, IC and IT dosing. For these studies, the Applicant made use of the fact that transgene expression levels (GFP) from AAV9.CB.GFP is indicative for the level SMN1 expression from OAV101, which are both under control of the same promotor. GFP expression of 55 ± 2.0%, 62.3 ± 2.4% and 79.6 ± 1.2% (n=3) for cervical, thoracic and lumbar regions, respectively was observed upon IT dosing of 1 x 1013 vg/kg AAV9.CB.GFP in one year old macaque fascicularis in Trendelenburg position. In mice, an ICV dose of 3.3 x 1014 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 macaque as compared to mice, although it should be noted that the age of the animals is not similar. ICV/IT treatment with AAV-CB.GFP revealed efficient transduction of motor neurons, also in the juvenile macaque monkeys. This would suggest that based on biodistribution data, also efficacy might be expected upon IT treatment of individuals at later age. However, as disease in older patients has progressed more as compared to younger patients, the occurred damage might not be reversible and later treatment would only prevent further deterioration.

Efficient transduction and partial amelioration of the SMA disease phenotype in the post-symptomatic setting was observed despite using the same AAV serotype for both induction of SMA phenotype at PND5 (scAAV9.shSMN) and expression of human SMN by administration of OAV101 at PND33-36. This observation is in line with published reports showing that pre-existing anti-AAV9 neutralizing antibodies have a minimal impact on transduction of AAV9 vectors administered IT in brain and spinal cord of NHPs (Gray et al., Gene Ther 2013;20:450-59; Guibinga et al. Mol. Ther. Methods Clin. Dev. 2024;32:101371).

Secondary pharmacology

To support the section of secondary pharmacology, the Applicant was asked to submit the secondary pharmacology study provided within the Zolgensma procedure EMEA/H/C/004750/0000 as well for this Itvisma application and discuss the expected influence of IT OAV101 treatment on diseased peripheral organs. The Applicant responded that the disease model in this study may have a more severe phenotype (especially in the periphery) compared to the currently indicated patient population. Data from that study may therefore not translate to the clinical treatment with Itvisma. If occurring at all, support for improvement of peripheral symptoms related to the disease should be collected from clinical data. This is agreed.

Pharmacokinetics

Analytical methods

For detection of anti-AAV9 immune responses, ECLIA and flow cytometry methods were developed and validated or qualified. Based on the available information, these methods seem to be adequate to measure the frequencies of AAV9 capsid-specific T cells in cynomolgus blood and the titres of anti-AAV9 antibodies in cynomolgus serum and CSF following in vivo treatment with OAV101.

Biodistribution of vector DNA and transgene mRNA (in murine and monkey tissues and body fluids) was evaluated with ddPCR and RT-ddPCR, respectively. However, it is not clear whether these methods were adequate and fit-for-purpose, as the methods were not formally validated. This is not in line with the Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014) indicating that PK studies should only use validated methods. However, the Applicant indicated that specificity and sensitivity of the methods were established and matrix interference was evaluated by spike/recovery experiments. For mouse tissue homogenates a LLOQ of 33 copies/reaction and for isolated gDNA a LLOQ of 9 copies/reaction has been indicated. For monkey tissue homogenates, plasma, blood cellular pellet, and CSF a LLOQ of 28 copies per reaction has been indicated. However, the amount of gDNA per reaction is not known. Furthermore, the Applicant indicated that vector genomes measured in the samples were normalized to endogenous CFTR, which was quantified in the same reaction using duplex ddPCR and served as a 2 copy reference gene. Normalization to CFTR allowed to present values of OAV101 as vg/diploid genome. However, for both ddPCR reactions (AAV vector DNA and CFTR) no LLOQ has been established relative to the amount of genomic DNA used in the reactions. Since both the LLOQ for the AAV vector genome and for CFTR were not properly determined, the ratio calculation may become unreliable, especially for the low-copy ranges. As such, the reliability of the distribution data in the PK and toxicity studies remained unclear. Especially the comparison between IV and IT biodistribution is relevant for the clinical consideration of the value of Itvisma compared to Zolgensma, thus requiring reliable non-clinical data. The Applicant was therefore requested to discuss whether their ddPCR and RT-ddPCR methods are fit-for-purpose to reliably determine vector DNA and transgene mRNA in blood and tissues in all GLP studies with biodistribution data (i.e. pivotal toxicity study and reproductive toxicity studies). In this discussion, the Applicant should thoroughly justify their chosen approach and provide further evidence that the measurements of the biodistribution studies in mouse and monkey fall within a range where reliable and reproducible data can be expected. Any available data on the specificity, sensitivity (LOD and LOQ), linearity/dynamic range and precision of the methods and the re-evaluation of the mRNA datasets (with reinterpretation of these data) should thereby be provided as well.

In the response related to the DNA ddPCR assay, it appeared that specificity of the assay was based on the relation with the titre assay (drug product) using the identical primer/probe set. Specificity in the titre assay was shown on the basis of lack of PCR signal in matrix-alone samples and in related products, but in samples of blood or tissue. As specificity may differ between sample types (e.g. due to presence of heme as PCR inhibitor or in case of low target DNA), the Applicant was asked to further discuss the specificity of the vector DNA ddPCR assay. In their response, the Applicant explained that the specificity of the vector DNA assay is shown by the data from the control groups, in which all but one sample from five studies were below the limit of quantification. It is agreed that these data from the control groups (with an almost 100% 'true negatives' in all sample types) indicate high specificity of the vector DNA ddPCR assay.

The recovery related to selectivity and matrix effect turned out to be 70-105%. Recovery distribution is usually acceptable in the 80-120% for Quality release tests. Although the current DNA ddPCR is not a release test, comparable criteria could apply. The skewed recovery distribution may indicate that values are somewhat underestimated by the assay. This may be predominantly relevant for the interpretation of data from tissue samples in which low vector transduction is desirable (e.g. gonads). The Applicant explained responded that the skewed recovery (70%) for vector was only observed in gonads and vector DNA in this tissue may be slightly underestimated. But despite that the true vector concentration in gonads may be somewhat higher compared to the measured concentration, vector DNA in gonadal tissue remains low compared to other tissues. As such, it is agreed that the skewed recovery does not impact the final conclusion on vector DNA levels in gonads compared to other examined tissues

Other assay parameters (sensitivity, dynamic range, precision) were determined and provided as well, as requested. Underlying reports were not provided. Based on the provided information, it seems that all vector DNA data above the LLOQ (28 copies/reaction for NHP, 33 copies/reaction for mouse tissue, 9 copies/reaction for mouse isolated DNA) and below the literature-based ULOQ (99.6% droplets) could be reliably determined. Only a few samples fell above the ULOQ, predominantly related to liver samples with high vector DNA content. Exclusion of these samples did not impact the conclusion of high vector biodistribution to the liver in both mice and NHP.

In the response related to the mRNA RT-ddPCR assay, it appeared that the specificity of this assay was based on the relation with the DNA assay, using the same primer/probe set and allowing specific detection of the vector-derived SMN protein via binding to the 5' UTR (part of the amplicon but not of endogenous SMN mRNA).

Initially, sensitivity (LLOQ) of the method was not established. This was an issue during the annual renewal of Zolgensma and the Applicant now provided the LLOQ (established with a statistical approach in line with ICH Q2, resulting in a dynamic LLOQ) and a summary of the re-evaluation of the mRNA datasets from the NHP studies. The amended tables in the study reports show that mRNA concentrations in the samples of the control group are below the limit of quantification and that the majority of DRG samples (in study 1970589) did not have sufficient quantity for analysis, as was mentioned by the Applicant before. In study 1970356 (timepoint 6 weeks), quantity of DRG samples was generally sufficient for analysis. As such, it is agreed that the interim evaluation time point provides information on mRNA expression in DRG compared to other tissues. Data on persistence of expression in DRGs (as measured at 12 months) is, however, limited.

The Applicant did not provide additional assay parameters for the mRNA assay, such as ULOQ, precision or matrix effect. Nevertheless, the Applicant explained that evaluation of additional parameters would require reference material (mRNA), but DNA reference material would also be possible according to literature. As for this mRNA assay the same primer/probe as in the DNA ddPCR method was used, parameters related to the ddPCR (determined with DNA reference material) would also be applicable to the RT-ddPCR. This is acceptable.

Although the Applicant has now shown that their DNA and mRNA PCR methods are fit-for-purpose, data on transgene expression in the pivotal NHP toxicity study are considered of limited value due to the lack of samples for some of the tissues and the high intragroup variability. Similar difficulties were also encountered in the non-GLP immune-suppressant study that evaluated the effects of prednisolone and rituximab/everolimus, respectively, on vector biodistribution and transgene expression. Consequently, the data do not allow any meaningful conclusions to be drawn about the impact of the tested immune suppressant regimen on the tissue distribution pattern or persistence of vector DNA or mRNA.

Biodistribution

Traditional ADME evaluation does not apply for ATMPs and therefore, only OAV101 biodistribution was evaluated in mice and NHP. This approach is agreed.

In one of the NHP studies, impact of pre-existing anti-AAV9 antibodies in serum on biodistribution of the vector to CNS and peripheral tissues was evaluated. However, as patients in clinical trials were required to have a baseline anti-AAV9 antibody titre of □1:50, the relevance of these NHP data for Itvisma patients is rather limited.

In the pivotal IT study in NHP, a dose-response was evident in most of the peripheral organs and tissues analysed, but absent in the CNS and DRG. The Applicant assumes that this might be due to a plateau that is achieved with the lowest dose tested (1.2 x 1013 vg). However, as no lower dose levels were tested, it remains unclear whether this assumption is indeed correct.

In the distribution comparison between IT and IV administration, considerable interindividual variability and lack of samples for several tissues (especially) in the IT study prevents drawing firm conclusions, predominantly with respect to (dose-dependent) SMN expression and persistence. In addition, potential decline in in transduction/expression level was observed with both administration routes, but whether this decline will continue over time is not clear. But persistence of SMN expression at the level that is reached shortly after administration is not supported by in vivo animal biodistribution studies. In addition, the absence of transgene mRNA data at final sacrifice prevents further insight in the potential relationship between SMN levels and observed toxicity in the animals, including the possibility for toxicity due to exhaustion of the protein expression machinery (as mentioned in the 2021 scientific advice EMA/SA/0000054059). Taken together, the value of IT administration above IV administration for SMN patients is questionable from a non-clinical biodistribution and toxicity point of view.

General toxicity

With respect to the pivotal GLP-compliant NHP toxicity study, no cellular responses to AAV9 nor immunogenicity to the transgene have been evaluated in this pivotal study. However, the data from non-GLP studies are sufficient in this respect, as immunogenicity of both the vector and human transgene is expected in NHP.

In this pivotal study, the Applicant could not establish a NOAEL, because the CNS and PNS pathologies already occurred at the lowest dose tested. A such, close monitoring of patients during the first period post-injection and via long-term follow-up will be essential and appropriate warning was added to SmPC section 4.4.

DRG toxicity

It can be considered that DRG toxicity is related to OAV101 treatment. Neuronal pathology has been shown in NHP for AAV(9)-related therapies in several publications (e.g. Hinderer et al., Hum Gene Ther, 2018; Hordeaux et al., Mol Ther Methods Clin Dev, 2018a and 2018b), and various AAV9 gene therapies carrying different transgenes and even shRNA expression constructs produce comparable T cell-rich DRG immune-inflammatory responses, suggesting that an immune response to the AAV9 capsid and/or vector genome is central in this tissue response. This AAV9 class effect is believed to be induced by high transgene expression leading to cellular stress due to an overabundance of the transgene product in the transduced cells (Hordeaux et al., Sci Transl, Med. 2020). Considering the use of a strong and ubiquitous promoter and direct administration of OAV101 into the CSF, neuronal toxicities were to be expected with this gene therapy. The DRG structure has an immune surveillance function with resident T and B lymphocytes, antigen presenting cells and ganglion satellite cells, which may enhance local susceptibility to AAV9-induced inflammatory changes. Literature further indicates that (severity of) DRG toxicity is at least dependent on the dose and the route of administration. Upon IT administration, the local vector concentration for DRG neurons is relatively higher than upon IV administration.

As DRG toxicity is observed from two weeks up to several months post-administration, involvement of the innate immune and adaptive system is expected. In one of the non-GLP studies, increased numbers of T cells in the DRG regions two weeks post-dosing correlated with immunoreactivity against the capsid and transgene expression, suggesting that initial adaptive immune responses may in part be driven by local exposure to the vector in individual DRGs. In addition, in DRG neuronal regions with high T cell infiltration also increased mRNA expression of the nucleic acid sensing RIG-I-like receptors was observed. This may imply a role for innate immune responses in DRG toxicity as well.

Absence of DRG toxicity findings in the murine studies and in the study in young male NHP may have several reasons. It could be the case that i) DRG toxicity was missed as DRG was not a target organ for microscopically histopathological analysis, ii) younger animals may be less susceptible to develop

DRG toxicity (e.g. Hordeaux et al., Hum Gene Ther, 2019), or that iii) inflammatory damage was reversed at the time of histopathological analysis and therefore not visible anymore (neuronal injury repair may also be more efficient in younger animals).

Based on the NHP toxicity study of OAV101 in combination with immune-suppressants, (anti-AAV9) systemic immune responses were considered not to be involved in the mechanisms of DRG toxicity and secondary consequences in the rest of the nervous system. Other potential mechanisms of DRG toxicity have not been investigated and therefore, the underlying cause(s) and the possibility that neuronal toxicity will also occur in patients remains unclear. Although DRG toxicity was partially resolved in the pivotal NHP toxicity study (at least with the lowest dose of 1.2x1013 vg/animal) and no clinical neurological observations were made in these animals (nor in the peripheral sensory nerve conduction velocity test in the animals from the non-GLP immune-suppressant study), safety data from several patients indicate that DRG toxicity is a realistic adverse event in human as well. Monitoring of patients for sensory neuropathy has been included in clinical trials and remains essential during follow-up and post-marketing.

Clinical relevance of the animal toxicity data

The murine toxicity studies and the safety study in young NHP were conducted with a pre-process A (research-grade) batch. As such, the relevance of the data for the clinic are considered limited.

In the other (distribution and) toxicity studies, the Applicant has generally used juvenile NHP. In the pivotal study, animals of 13-19 months of age would resemble a developmental human age of ≥3 years. The Applicant did not further explain the use of juvenile animals, while the proposed human target population consists of patients ≥6 months of age. As such, the NHP (used in the pivotal study) and patients may differ in the development and maturation of several (AAV9-transduced) organ systems. The impact of the use of an NHP juvenile age group on the susceptibility for e.g. CNS toxicity compared to patients was not further discussed. Therefore, the translatability of the NHP toxicity -and biodistribution- data to (especially very young) patients that are IT injected is not clear.

Furthermore, part of the monkeys used in the non-clinical studies showed a humoral and/or cellular immune response against the vector capsid prior to dosing of OAV101. In contrast, patients are preferably AAV9-naïve. This difference in immune status may have contributed to the relatively mild systemic response (related to liver enzymes, thrombocytes, etc.) post-dosing of OAV101 in NHP, while some patients developed severe hepatotoxicity or thrombocytopenia, although species specificity of these findings cannot be excluded. Whether this difference in immune status may also have affected the neurotoxic response (and thus the conclusions regarding the mechanism behind DRG toxicity and the translatability of the NHP data to the clinic) and the response towards the immunosuppressive regimen remains unclear.

Clinical dose determination

Considering that a NOAEL could not be established in the pivotal single dose toxicity study, no safety margins have been provided either. Nevertheless, the Applicant has decided to use the fixed dose of 1.2x1014 vg in patients (Phase III), irrespective of their age or weight. This clinical dose was based on minimal efficacy data, i.e. >40% lumbar spinal cord motor neuron transduction and SMN expression to improve disease phenotype in the SMA mouse model (although actual efficacy data, such as survival and locomotor activity, in the SMNΔ7 mice have not been taken into account). And it was based on the achievement of >55% transduction of the spinal cord neurons in healthy NHP at a dose of 1.2x1013 vg IT. Assuming that CSF volume in NHP would be 10-fold lower than in patients, a clinical dose of 1.2x1014 vg IT would result in effective transduction of target tissue. This assumption of CSF scaling is based on data from Sullivan et al. (J Transl Med, 2020), in which mean CSF volume of adult cynomolgus monkeys (mean 3.6 kg weight) was determined to be around 12 mL. However, in the non-

clinical toxicity studies, juvenile animals were used, which have lower body weight (less than 2.5 kg in pivotal study) and potentially also lower CSF volume. This would result in >10-fold difference in CSF volume between juvenile monkeys and young children (CSF volume around 120 mL) and patients ≥2 years (CSF volume around 150 mL). And the human equivalent dose of the NHP dose of 1.2x1013 vg would not be the same for the different age groups. Moreover, as mentioned before, the juvenile animals used in the non-clinical studies represent the developmental stage of a young child (≥3 years of age). One NHP study was conducted in animals during their infancy (4-6 months of age). However, this study was conducted with a pre-process A batch, without GLP compliance and only using male monkeys. The clinical relevance of the obtained data is therefore limited, and these data cannot be used as support for the clinical dose determination. Taken together, the animals used in the studies relevant for clinical dose determination are not fully representative for the human target population (≥6 months of age), with respect to (CNS) development as well as CSF volume.

As biodistribution in NHP plateaued at 1.2x1013 vg, it was considered that higher doses would not lead to additional spinal cord transduction. In addition, central and peripheral nervous system toxicity at this dose would be (partly) reversible and non-progressive in the pivotal NHP toxicity study, in contrast to the higher doses 3x1013 and 6x1013 vg. It indeed appears that increasing the dose will not considerably increase the transduction efficiency in nervous tissues, however, the number of animals evaluated in the pivotal NHP study is limited and there is quite some interindividual variation and no dose lower than 1.2x1013 vg was tested (see PK discussion).

Genotoxicity

It can be agreed with the Applicant that based on the low integration risk and the risk management plan in place for OAV101, which includes long-term follow-up studies that specifically include the occurrence of new malignancies and tumours, no genotoxicity studies are needed.

Carcinogenicity

It can be agreed with the applicant that the potential risk for OAV101 mediated tumorigenesis is low and remains currently theoretical. Long-term follow-up studies that specifically include the occurrence of new malignancies and tumours as part of the risk management plan in place for OAV101. Therefore, tumorigenicity studies are not needed.

Reproduction Toxicity

FEED - studies

Since scAAV9 vector DNA and transgene expression was detectable in gonads, the applicant conducted a male (2470261) and a female (2470188) FEED study in mice. In this species, distribution to germ cells (sperm and oocytes) has not been detected before. The studies did not evaluate the risk for germline transmission but rather evaluate the effect of potential transduction of gonadal tissue on fertility and the ability to reproduce. Males were treated IV with 1.1 x 1013 vg/kg and 1.1 x 1014 vg/kg, respectively and mated 4 weeks later with untreated females. Females in Study 2470261 were similarly dosed as the males but mated 2 weeks postdose with untreated males. In both studies and thus in both sexes, no effect was observed on the fertility fecundity, mating indices, uterine examination data, or embryonic development of IV treatment with OVA101 in mice. Although no vertical transmission of OAV101 vector DNA was observed in foetuses, it is important to note that these mouse studies are not appropriate to further evaluate the risk of inadvertent germline transmission, since mice did not reveal any vector DNA in germ cells.

In study 2170026 distribution of IV or ICV administered OAV101 at PND1 to germ cells (sperm and oocytes) have not been detected, despite presence of the vector in ovaries and testes. As upon IV and ICV dosing of OAV101 to mice the vector seems not to transduce oocyte and sperm cells, the

relevance of the FEED studies and the ongoing EFD study in this same species for germline transmission is thus debatable. IV treatment with OAV101 could be regarded as a worst case scenario for IT treatment as for Itvisma a lower clinical dose is used (compared to IV treatment with OAV101 in Zolgensma) and as distribution to periphery in general, and gonadal tissue more specifically here, is expected to be lower upon IT compared to IV treatment.

Other Toxicity studies

Vector distribution to gonads was observed in both mice and monkeys and further investigated by the applicant.

Mice

In study 2170026, the Applicant evaluated the potential for germline transmission and integration in germ cells upon administration of OAV101 of a single IV or ICV injection at doses of 1.1 x 1014 vg/kg or 3.3 x 1013 vg/kg administered to neonatal FVB/NCrl mice on PND1. The ddPCR results showed overall low level of biodistribution of OAV101 in mice to the gonads, but absence of OAV101 in sperm. Molecular localization analysis by in situ hybridization (ISH) did not reveal OAV101 vector DNA within the seminiferous tubules in testis or oocytes in ovary, while some interstitial cells in testis and some granulosa and ovarian stromal cells turned out positive in ovary. Thus although some specific cell types in the gonads are positive for OAV101 DNA, the vector did not transduce germ cells. Results from both methods are consistent, also with literature where AAV9 vector DNA was not detected in seminiferous tubules of the testes or oocytes of mice by ISH following local or systemic administration (Zhao et al, 2020).

The relevance of this study for the IT route of administration is not so clear but might be best comparable to ICV administration, as in both cases, the vector is more locally administered. It might be the case that ICV is even more local than IT and that in the latter case, higher vector levels will come systemic, however, that was not investigated here. Therefore, the relevance of this ICV study with regard the risk for vector presence and integration in gamete cells upon IT application in human is difficult to determine. It can be concluded though that in mice upon IV and ICV dosing, vector seems not to transduce oocyte and sperm cells. As such the relevance of the FEED studies and the ongoing EFD study in mice are debatable.

Monkey

A molecular localization study on gonadal tissues was undertaken to evaluate and characterize the transgene expression (GFP or mCherry) using immunohistochemistry for the reporter transgene products green fluorescent protein (GFP) in ovary and GFP or mCherry in testes from sexually mature cynomolgus macaques which were sacrificed 28 days post-dosing. In male animals dosed with scAAV9-CB-GFP vector at 3 x 1013 vg/animal by the IT route or dosed with scAAV9-CB-mCherry vector at 7 x 1013 vg/kg by the IV route, no GFP or mCherry transgene was detected (by immunohistochemistry) in the seminiferous tubules or sperm cells of these males. The IT dose is relatively higher compared to the proposed clinical dose for Itvisma, but the IV dose is lower as compared to the clinical dose for Zolgensma treatment which might indicate that males treated with Itvisma might be not at risk. Although it should be noted that male patient treated with Itvisma might be younger than the animals treated in this study.

In cycling cynomolgous monkey aged 13-17 months (body weight 1.2 to 1.8 kg) that were dosed with 1.0 x 1013 or 3.0 x 1013 scAAV9-CB-GFP by the IT and ICM route in Study 2070139, transgene protein (GFP) was detected in a dose dependent manner in a subset (up to 10%) of oocytes and ovarian stromal cells by either route at 1.0 x 1013 or 3.0 x 1013 vg/animal indicating transduction of female germ cells with this vector under the experimental conditions. It should be noted that these juvenile female cynomolgus monkeys would normally be regarded sexually immature. According to the

applicant, the animals were already cycling, determined by evidence of Graafian follicles in ovaries. The observation that oocytes can be transduced with the AAV9 vector is different from the observation in the study 2170026 (IV or ICV administration to neonatal mice) where a slightly different vector was used next to a different animal species and age at dosing. The NHP study report indicates that the transgene (GFP) expression in oocytes and ovarian stromal cells reflects a sex-related difference in transgene expression that was not previously reported but does not further explain this different finding nor discusses the relevance of this finding for human.

For Itvisma evaluation, NHP is regarded a species closer to human based on CNS / PNS similarities and genetic closeness. Because of the latter argument it is also expected that the transduction process in NHP, unlike in mice, might be more similar to human. However, how specific characteristics of gonads / gametes in NHP and mice compare to human remains unclear. It is for instance not clear whether the barrier around an oocyte or the ripening of oocytes is comparable for all species. This might influence the human relevance of the findings. More investigation / evaluation as well as fundamental knowledge on the cellular characteristics of primordial follicles, primary follicles, and oocytes in different stages of development would therefore be needed. As these structural and functional characteristics of gonadal (germ and accessory) cells are likely to influence the level of transduction of oocytes, knowledge about these characteristics and processes for the different species used may inform on the human relevance of the findings in NHP.

Detection of GFP expression in oocytes following administration of scAAV9-CB-GFP to female monkeys is indicative for OAV101 transduction of oocytes in NHP. At that stage it is not known whether also integration of the vector DNA in oocytes does occur. These data were also discussed recently in connection with PAM-024 (LEG) for Zolgensma. During this discussion, the applicant indicated that assessing whether or not the AAV vector is integrating into oocytes in female NHP is very challenging due to the rather low frequency of oocytes that were transduced (up to 10%) and the estimated low frequency of AAV vector genomic integration that is usually ranging between 0.01% to 1%. As a consequence, large numbers of monkeys would be required in order to detect potential oocytes with vector integration. Furthermore, the methods that could be used to address genomic integration in oocytes are not sufficiently sensitive for detecting integration on a single cell level. Based on these difficulties, it was acknowledged that there is currently no possibility to address the risk of inadvertent germline transmission further by evaluating whether or not OAV101 or a surrogate vector is integrating into the genome of oocytes. Reopening of this discussion is not intended, as it is not expected that further discussion would result in a different conclusion. Instead, it has to be acknowledged that OAV101 genomic integration in oocytes and the likelihood of inadvertent germline transmission can currently not be further evaluated. Accordingly, information that the scAAV9 viral vector used in OAV101 was shown to transduce oocytes in NHP has been included in Section 5.3 of the SmPC.

Apparently, AAV9 can transduce oocytes, but integration into the genome was shown to be rare or limited (Jakob et al 2005, Kanatsu-Shinohara et al 2022, Fonck et al 2023, Borges et al 2024). In case transduction results in epigenomic presence of the AAV vector in the fertilized egg, rapid dilution will occur upon cell division, and therefore the vector will likely not be detectable in the offspring. In addition to that, germline transmission events for AAV have not been observed in many studies involving rodents and NHPs. Therefore, it can be concluded that on top of the low levels of vector observed in gonadal tissues, the structure and developmental characteristics of oocytes as well as the limited or rare level of integration upon transduction, will limit the risk of germline transmission even further. However, it is still not entirely clear whether the observed AAV9-mediated transduction of oocytes in NHP is likely to occur in human or not. This is acceptable.

Toxicokinetics and exposure margins

For Itvisma, tissue biodistribution of the vector and transgene is more relevant for (potential) toxicity than systemic exposure. For this IT-based gene therapy, distribution to both central and peripheral tissues was observed. No NOAEL could be determined in the pivotal toxicity study in NHP and therefore no safety margin to clinical 'exposure' was available.

Developmental and reproductive toxicity

Data from male and female FEED studies as well as from an EFD study do not indicate any OAV101 related toxicological effects with regard to fertility, (early) embryonic and foetal development. As such, for section 4.6 of the SmPC, a separate section on contraception was not considered to be needed. This is reflected in the SmPC.

Environmental risk assessment

The ERA contains an overview of the AAV viral vector. The plasmid maps of the pSMN plasmid and both required helperplasmids are presented in the ERA module (module 1.6.2). Information on the presence of replication-competent AAV (rcAAV) and the absence of rcAAV detection in drug substance batches is summarized in module 1.6.2. The potential presence of rcAAV in OAV101B as a contaminant, or as an indirect consequence of homologous recombination with wild type AAV and OAV101B in vivo, is not considered to constitute an environmental risk, as this rcAAV is similar to wildtype AAV, which is already globally endemic and a non-pathogenic virus by nature. Moreover, the drug substance is tested as part of manufacturing and should be negative for rcAAV.

The plasmid master cell bank (MCB) has been fully characterised. Plasmid identity is confirmed by restriction digest and Sanger sequencing. The molecular characterisation of OAV101B has been performed adequately.

The amount of vector that is shed by patients is transient and peaks in the days after administration after which it declines. The level to which individuals other than the patient will be exposed in worst case is low, and orders of magnitude lower than the amount of particles administered to the patient. Considering the characteristics of the vector and the transgene, hazardous effects of these particles in the amount that third individuals are exposed to in a worst case scenario are not expected. Further spread of these particles in the environment is considered to be limited. The statement that no precautions for handling human waste are warranted for OAV101 was agreed upon. Statements in the SmPC regarding measured to minimize the likelihood of spread in the environment or to non-target individuals are considered appropriate.

4.4.2. Conclusions

Pharmacology

There is sufficient rationale for IT treatment with OVA101B in older patients. However, whether efficient transduction of the motor neurons might overcome the already caused damage cannot be predicted.

Pharmacokinetics

Reliability of the PK data are being hampered due to the lack of samples for some of the tissues, high intragroup variability, and low animal numbers. Methods to evaluate biodistribution in animals have not been formerly validated, but the adequacy of these methods to reliably measure vector DNA and transgene mRNA has been shown.

Biodistribution data in animal species indicate that injection of OAV101 in the spinal cord results in considerable vector transduction and transgene expression in the neuronal tissue, including DRGs.

Despite local administration, substantial distribution to peripheral tissues was also observed (which can be considered positive in case of peripheral manifestations of the disease), although lower than with IV administration. The type of tissues involved in this distribution was comparable between IT and IV administration, but with IT injection the extent of CNS and PNS tissues involved in OAV101-related toxicity was higher. Taken together, from a non-clinical toxicity and biodistribution point of view, the value of IT administration above IV administration is limited and rely on clinical data.

General toxicity

Single dose toxicity data indicate inflammation and degeneration in neuronal tissue following IT administration of OAV101. Although the clinical relevance of the NHP data is not fully clear and considerable individual variability was observed within the data, the current non-clinical studies are considered sufficient to indicate which toxicity may be expected in patients following IT administration and thus should be monitored and managed.

Reproduction toxicity

There seems to be no negative influence of IV treatment with OVA101 of male and female mice on fertility, embryonic and foetal development. Germline transmission seems not likely based on a study in mouse, but a study in NHP might indicate that, as treatment with AAV9 vector encoding GFP resulted in transgene inside germline cells. The relevance of these data for treatment with OAV101 is not clear. The SmPC therefore recommends a thorough benefit-risk evaluation prior to treatment of women who are pregnant ot may become pregnant.

Environmental risk assessment

The risk to the environment that is related to the use of OAV101 following IT administration is considered negligible. Therefore, it is agreed that an environmental monitoring plan for Itvisma is not considered needed.

5. Clinical aspects

5.1. Introduction

5.1.1. Good Clinical Practice (GCP) aspects

All clinical studies were conducted in compliance with GCP, as claimed by the Applicant. Four Health Authority site inspections were conducted for study B12301.

Full reports of these GCP inspections were provided by the Applicant, no critical issues were found in any of these inspections.

A routine EMA GCP inspection was conducted by the Health and Youth Care Inspectorate and the Danish Medicines Agency in the South African site, the Vietnam site and the sponsor site of study B12301. The outcome of this inspection has been provided.

At the EMA inspection of the investigator site in South Africa, there were 8 major findings (no critical findings). At the EMA inspection of the investigator site in Vietnam there were 12 findings, 10 major and 2 minor (no critical findings).
At the EMA inspection of the sponsor site, Novartis AG (Basel, Switzerland), there were 14 findings, 4 critical findings and 10 major findings.

Overall, the reported critical findings indicate serious, and in some cases structural, inadequacies of several aspects of the clinical trial conduct. The Applicant was asked to address all the reported critical and major findings with adequate corrective and preventive actions (CAPA). The provided CAPA were considered generally adequate by the EMA inspection team.

The inspection team concluded that the reported findings did affect, to some extent, the quality of the data. In addition, as a result of the number of major and critical findings on relevant GCP aspects, the GCP-compliance was considered impacted. However, the setup of the trial and especially the nature of the primary, and most relevant secondary, efficacy parameters and the way/processes by which these were collected (independent evaluations by blinded experts), is likely to have 'reduced' the impact on the reported core data. Therefore, the inspection team is of the opinion that, from the GCP perspective, the (core) trial results can be used in this procedure.

5.1.2. Tabular overview of clinical trials

Table 5: Tabular overview of main clinical studies

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
B12301	Enrolment completed. FPFV: 01-Feb-2022 Treated 126 participants / 125 planned	Randomized, double-blind Sham control	Study drug: OAV101B $1.2x10^{14}$ vg administered IT Control: Sham procedure	Diagnosis of SMA, age 2 to <18 years, treatment-naïve, sitting and never walked
B12302	Enrolment completed. FPFV: 12-Jan-2023 Treated 27 participants / 28 planned	Open-label, single arm	Study drug: OAV101B $1.2x10^{14}$ vg administered IT	Diagnosis of SMA, age 2 to <18 years, discontinued nusinersen or risdiplam, sitting and never walked
CL-102	Enrolment completed. FPFV: 21-Dec-2017 Treated 32 participants / 51 planned	Open-label, dose ranging	Study drug: OAV101B $6.0x10^{13}$ vg, $1.2x10^{14}$ vg or $2.4 x10^{14}$ vg administered IT	Diagnosis of SMA, age 6 to <60 months, treatment-naïve, sitting and never walked
LT-002	Enrolment completed.	Long-term follow-up study	None	Received OAV101 in a clinical study; enrolled participants treated in CL-102
A12308	Enrolment ongoing.	Long-term follow-up study	None	Received OAV101 in a clinical study; enrolled participants treated in B12301 and B12302

Abbreviations: IT=intrathecal;

Source: SCE-Section 1.2, study protocols

5.2. Clinical pharmacology

OAV101B is a viral gene therapy product to be administered intrathecally. Conventional clinical pharmacokinetic studies are not possible for a viral gene therapy product. OAV101B will behave as an AAV9 virus. In humans, the shedding into excreta and the immunogenicity is investigated. Also the amount of SMN protein in blood was investigated. The presence of viral vector in blood (to estimate the amount distributed from the CSF to the systemic circulation) was not investigated. Other pharmacokinetic studies for the viral vector are not feasible.

OAV101B is intended to be administered as a single dose of 1.2×10^{14} vector genome copies in paediatric patients. To date, the clinical development program for OAV101B includes 3 clinical studies in paediatric patients (studies AVXS-101-CL-102, COAV101B2301 and COAV101B2302).

5.2.1. Methods

Analytical methods

Droplet digital polymerase chain reaction (ddPCR) was used to measure shed virus. Three shedding assays were used throughout the clinical development of OAV101B. An ddPCR assay was developed to support study AVXS-101-CL-102 (report RPT-270). Subsequently, another ddPCR assay was developed and validated and cross-validated to support studies COAV101B2301 and COAV101B2302 (reports DMPK R2000473-pk-01 and DMPK R2100861-pk). The key difference between these assays is sensitivity. The assays showed similar sensitivity, whereas the PPD assay is less sensitive. The adaptive immunological responses to OAV101B were monitored in the 3 clinical studies using different analytical techniques: ELISA or ECLIA for anti-AAV9 antibodies, ELISpot for T-cell-mediated immunity to AAV9, ELISA or ECLIA for anti-hSMN antibodies and ELISpot for T-cell-mediated immunity to hSMN.

5.2.2. Pharmacokinetics

Absorption + biodistribution

Following intrathecal administration, the absorption at the target site is 100%. Biodistribution to the systemic circulation is needed to transfect all tissues affected by the disease. The biodistribution into the systemic circulation following intrathecal administration was not investigated. It remains therefore unknown how much of the administered dose is able to biodistribute into the systemic circulation and is able to biodistribute into tissues. Based on the provided shedding data, the systemic exposure to OAV101B is most likely ~70-fold lower than following IV administration. No increase in SMN protein levels were observed following intrathecal administration of OAV101B.

The CSF volume increases within the first 1 to 2 years after birth based on literature, after which growth slows and volume remains steady into adolescence In subjects aged 6 months to 2 year, the CSF increases between 1.0- to 1.6-fold. Based on this, the exposure in paediatric patients aged 6 months to <2 years old may be a factor 1.6-fold higher compared to patients aged ≥2 years. Based on the limited data in the patient subgroup aged 6 months to <2 years, the safety profile appears comparable with the children aged ≥2 years. This indicates that the higher exposure might not be clinically relevant, but robust conclusions cannot be drawn.

Shedding

Shedding of OAV101B was observed in faeces, urine, saliva and nasal swab. Shedding of OAV101B is primarily via faeces and the vast majority of the dose is shed within 1 month after dose administration. Peak shedding in faeces was estimated to be between 0.005% to 0.03% of total dose.

Immunogenicity

As expected, an immunological response was observed for AVV9 after intrathecal administration of OAV101B. This is expected to be transient. No immunological response was observed for hSMN in most patients. Anti-SMN antibodies were only observed in 2 subjects at the lowest reportable titre (1:10).

5.2.3. Pharmacodynamics

5.2.3.1. Mechanism of action

SMA is a monogenic disorder, caused by homozygous deletions/mutations of a single gene, *SMN1*, at 5q13. Transcription of *SMN1* produces full-length mRNA transcripts that encode the SMN protein, which is crucial for survival and function of motor neurons in the brain stem and spinal cord. Consequently, depletion of the SMN protein in SMA leads to denervation atrophy of the skeletal muscles.

Onasemnogene abeparvovec is a non-replicating recombinant AAV vector that utilizes AAV9 capsid to deliver a stable, functional human SMN transgene. The *SMN1* gene present in onasemnogene abeparvovec is designed to reside as episomal DNA in the nucleus of transduced cells and is stably expressed in post-mitotic cells. Expression of the transgene is driven by a constitutive promoter (cytomegalovirus enhanced chicken-β-actin-hybrid), which results in continuous and sustained SMN expression. Proof of the mechanism of action (MoA) mainly derives from non-clinical studies. The AAV9 virus is not known to cause disease in humans.

5.2.3.2. Primary and secondary pharmacology

The mode of action of OAV101 or its effect on the desired therapeutic target cannot be measured clinically.

No designated PD studies were carried out for OAV101B. No validated PD markers of SMA drug response are reported in the literature.

SMN protein levels were measured in peripheral blood for potential PD assessments in both phase III studies (B12301, B12302). No notable changes in mean SMN protein levels in peripheral blood were observed in both studies following OAV101B administration. No considerable differences in mean SMN protein level in peripheral blood has been documented in Study B12301 in the OAV101B arm compared to the Sham arm.

No secondary pharmacology studies have been conducted for OAV101B.

5.2.3.3. Pharmacodynamic interactions with other medicinal products or substances

Pharmacodynamic interactions with other medicinal products have not been studied.

5.2.3.4. Genetic differences in PD response

There are no known genetic differences impacting the response to OAV101B.

5.2.3.5. Immunological events

Adaptive (humoral) and innate immune responses against OAV101B were examined in patients.

Humoral immunogenicity (serum and CSF anti-AAV9 antibody, serum anti-SMN antibody) was assessed in the Phase I/II dose-ranging Study CL-102, and in the Phase III studies B12301 and B12302. Pre-dose CSF anti-AAV9 antibodies were assessed in B12301 and B12302 studies.

Innate immunogenicity was assessed by measuring biomarkers of complement activation in studies B12301 and B12302.

Anti-AAV9 antibody response

Baseline serum anti-AAV9 antibody titers were below or equal to 1:50 in all participants treated with OAV101B in studies CL-102, B12301, and B12302. Anti-AAV9 antibody titers increased in all OAV101B-treated patients, and the titers remained elevated at the end of study (or last sampling timepoint). In Study CL-102, serum anti-AAV9 antibody titers reached up to 1:3200 at last sampling timepoint (Day 30 post-dose). In studies B12301 and B12302, median serum anti-AAV9 antibody titers reached or exceeded 1:819200 at Week 52.

Pre-dose CSF anti-AAV9 antibodies were negative for all patients in studies B12301 and B12302.

There was no clear association of post-dose serum anti-AAV9 titers with safety or efficacy.

Anti-SMN antibody response

In Study CL-102, no anti-SMN antibody response was observed in any participant (measured by ELISA).

In studies B12301 and B12302, anti-SMN antibody response was measured by an assay with ECLIA format, more sensitive than ELISA.

None of the participants in Study B12301 tested positive for pre-existing anti-SMN antibodies. In Study B12302, 1/27 participants (3.7%) tested positive for pre-existing anti-SMN antibodies with titer of 1:10.

In Study B12301, 5/75 (6.7%) of the participants in the OAV101B arm were positive for anti-SMN antibody at one or more timepoints in Study B12301. In Study B12302, 2/27 (7.4%) participants were positive for anti-SMN antibody at one or more timepoints. Titers in both studies ranged from 1:10 to 1:80. At Week 52, 2 participants in Study B12301 were positive for anti-SMN antibody with titers of 1:10 and 1:20, whereas no participants in Study B12302 were positive.

There was no clear association of positive anti-SMN antibody responses with safety or efficacy.

Complement panel

In Study B12301, complement panel was assessed prior to dosing, and post dose on Day 2 and Week 1, 2, 4, 12, and 52. In Study B12302, complement panel was assessed prior to dosing, and post-dose on Day 2, and Week 1, 2 and 4. Complement panel included C3, C5b-9, CH50 and Factor Bb.

In Study B12301, changes from baseline to Week 52 in biomarkers of complement activity were observed in both the OAV101B and the Sham arm. The changes observed in the OAV101B arm from baseline to Week 12 are indicative of complement activity with a peak activation at Week 1 characterized by high C5b-9 values (Median of 395 µg/L >ULN).

In Study B12302, changes from baseline to Week 4 in the complement panel (C3, C5b-9, CH50, Factor Bb) were observed following OAV101B administration. The changes observed are indicative of complement activation by Week 1 characterized by a transient increase in C5b-9 values.

T-cell mediated responses to AAV9 and SMN were assessed in study CL-102, however without collection of pre-dose baseline samples.

5.2.4. Pharmacokinetics/pharmacodynamics (PK/PD)

OAV101B exposure and SMN protein expression from OAV101B in motor neurons of the spinal cord is the driver of its PD effect. Determining OAV101B exposure in CNS tissues is not possible because it is not ethical to sample. Moreover, systemic exposure of an AAV-based gene therapy such as OAV101B does not reflect its exposure in the CNS tissues, as gene therapy vector clears quickly from the blood compartment, while it persists in the CNS tissues. In addition, SMN protein concentration in peripheral blood cells does not reflect SMN protein concentration in the CNS tissues in general or in spinal motor neurons in particular, and therefore may not correlate with measures of motor function. Therefore, exposure-response relationship cannot be assessed.

5.2.5. Dose selection and therapeutic window

The proposed dose of OAV101B is a single IT dose of 1.2×10^{14} vector genomes (vg) for the entire initially proposed patient population (SMA patients aged 6 months and older). This dose was primarily informed by nonclinical studies, and subsequently evaluated in clinical study CL-102, which is described and discussed in section 6.3.1.

5.2.6. Overall discussion and conclusions on clinical pharmacology

5.2.6.1. Discussion

Pharmacokinetics

OAV101B in Itvisma is a viral gene therapy product to be administered intrathecally. Conventional clinical pharmacokinetic studies are not possible for a viral gene therapy product. The pharmacokinetics of OAV101B have been evaluated in a limited number of PK studies. The limited number of PK studies is acceptable for a viral vector.

Droplet digital polymerase chain reaction (ddPCR) was used to measure shed virus. The adaptive immunological responses to OAV101B were monitored using different analytical techniques: ELISA or ECLIA for anti-AAV9 antibodies, ELISpot for T-cell-mediated immunity to AAV9, ELISA or ECLIA for anti-hSMN antibodies and ELISpot for T-cell-mediated immunity to hSMN. The analytical methods seem sufficiently validated to accurately determine to determine the shed virus and the immunological response in patients after OAV101B administration.

The Applicant did not investigate the biodistribution into the systemic circulation following intrathecal administration. It remains therefore unknown how much of the administered dose is able to biodistribute into the systemic circulation and is able to biodistribute into tissues. Therefore, the systemic exposure in paediatric patients is solely based on literature data on the CSF volume.

Shedding of OAV101B was observed in faeces, urine, saliva and nasal swab. Shedding of OAV101B is primarily via faeces. The maximal shedding concentration in faeces was 70-fold lower following intrathecal administration of 1.2×10^{14} compared with IV administration of 1.1×10^{14} vg/kg (study AVXS-101-CL-302). Shedding via stool was reported and stated in the SmPC.

An immunological response was observed for AVV9 after intrathecal administration of OAV101B. The effect on Efficacy and Safety is discussed in 6.3 and 6.4, respectively.

Pharmacodynamics

Onasemnogene abeparvovec addresses the root genetic cause of SMA by introducing a functional copy

of *SMN1* in the transduced cells, encoding the SMN protein. Although the SMN protein is ubiquitously expressed in all tissues, its expression is particularly crucial for survival and function of motor neurons in the brain stem and spinal cord. The IT formulation of OAV101B enables delivery into the CSF of the spinal cord, hence (almost) directly to the site of action. The MoA of OAV101B is SMA-specific, and has been described adequately in section 5.1 of the SmPC.

The direct therapeutic effect of OAV101B on the main therapeutic target cannot be measured clinically, as direct measurement of the SMN protein levels in second motor neurons in patients is not possible. The SMN protein is intracellular, and not secreted by motor neurons, hence measurement of the SMN protein in the CSF or peripheral blood would be of doubtful interpretation (as a probable result of cell lysis), and therefore are not considered meaningful PD markers.
While the primary pathology of SMA is motor neuron degeneration and muscular weakness, which is targeted by the MoA of OAV101B, SMN deficiency has widespread effects in other tissues, leading to a range of non-neurological symptoms. Literature reports the involvement of multiple organs in SMA, including the heart, liver, pancreas, intestine, kidneys, spleen and immune system, in addition to the nervous system, suggesting that SMA should probably be better regarded as a multi-system disorder. Dysfunctions of peripheral tissues and organs are more relevant and prevalent in the more severe forms of SMA, particularly type 1, which -prior disease-modifying treatments- were often overshadowed by early mortality. With the advent of disease-modifying treatments, systemic impairments may become more relevant in these patients, as Zolgensma extends the life expectancy. Systemic impairments may become significant also in milder forms of SMA over time, and even become the primary concerns.
As the CSF is absorbed into blood, a proportion of OAV101B will reach the systemic circulation. This may result in off-target toxicities, but possibly also in systemic therapeutic effects with potential clinical relevance. The systemic distribution of OAV101B is informed by the performed NPH studies, showing OAV101B mRNA expression in the heart, liver and muscles, with lower concentrations than those after the IV administration. Therefore, the IT formulation does distribute systemically in animal models, but to a lower extent than the IV formulation (equivalent of Zolgensma). There is no clinical PK data comparing systemic exposure following IT versus IV administration. However, based on the shedding data reviewed, systemic exposure to OAV101B in humans is estimated to be ~70-fold lower than following IV administration. How this may translate into potential clinical effects (if any) in peripheral organs is unknown, since these aspects were not investigated in the clinical studies. This would have been of relevance for the treating physicians, for informing decisions on the most suitable formulation for their patient (besides the feasibility/suitability of the administration route per se), in case the patient was eligible for both. As discussed in scientific advice EMA/SA/0000054059, the IT administration may not be the optimal treatment option for patients with relevant visceral involvement (such patients with SMA type 1).
No drug-drug interactions are expected for OAV101B, hence no dedicated studies are considered necessary. No genetic factor is known, or hypothesised to, affect the PD properties of OAV101B.

Immunological events

The adaptive (humoral) and innate immune responses against OAV101B were examined in patients.
The humoral immune response to OAV101B was assessed in studies CL-102, B12301 and B12302, by measuring serum antibodies against the viral vector (anti-AAV9 antibodies) and against the transgene (anti-SMN antibodies) before and after dosing. Anti-AAV9 antibodies in the CSF were assessed prior to dosing, and were negative for all patients.
Per protocol, patients were *excluded* in case of serum anti-AAV9 antibody titers >1:50 or elevated at screening (possible implications of this exclusion criterion are discussed in the clinical efficacy section). As a results, at baseline, all participants had serum anti-AAV9 antibody titers below or equal to 1:50. Only 1 participant from Study B12302 (n=27) tested positive for anti-SMN antibodies prior to dosing

(titer of 1:10). Of note, study B12302 was conducted in patients previously treated with SMN-targeted treatments (nusinersen or risdiplam), which may explain pre-existing anti-SMN antibodies (the *SMN1* and *SMN2* genes are almost identical).
One day prior to treatment and for the following 30 days, OAV101B treated patients received prophylactic immunomodulatory therapy with prednisolone 1mg/kg/day.
Post-dosing, anti-AAV9 antibody titers increased in all OAV101B treated participants, and remained elevated at week 52, with median titers ≥1:819200. Seven patients in total (5/75 in the OAV101B treated arm in Study B12301, and 2/27 in study B12302) were positive for anti-SMN antibody at one or more timepoints, with 2 patients being positive at week 52 (titers of 1:10 and 1:20).
Therefore, the humoral immune response to OAV101B is primarily mounted against the capsid. The SMN protein is endogenous, and transcribed from the *SMN2* gene in SMA patients, although in small amounts, hence no substantial immune response is mounted against the transgene.
The innate immune response was assessed by measuring complement biomarkers before and after dosing in studies B12301 and B12302. In both studies, there was complement activation following OAV101B administration characterized by transient increase at Week 1 in mean C5b-9, which is the final product of the complement cascade and a marker of total complement activity. Activation of the complement system is potentially leading to adverse effects, such as thrombotic microangiopathy, hepatotoxicity, reduced gene therapy efficacy. While some level of complement activation was observed, the AE profile was not severely impacted – no cases of thrombotic microangiopathy, reduced hepatotoxicity. Complement activation as an early response to OAV101B did not impact the change in total HFMSE score observed at the end of Follow-up Period 1 (Week 52). Yet, as OAV101B is injected intrathecally, it is not clear how measurement of serum immunological markers (complement and antibodies) reflects possible immune responses in the CSF. The Applicant indicated that antibodies in the CSF (are believed to) arise from diffusion from blood. The rate of clearance of IgGs from the CSF is more rapid than the rate of diffusion of the IgGs from blood into the CSF (Noguchi et al., 2017). Consequently, the titers of anti-AAV9-Abs are expected to be significantly lower in the CSF than in blood. However, as for OAV101B CSF anti-AAV9 antibodies were measured only prior to dosing but not after, there is no data to substantiate immunogenicity of the product at the site of administration.

5.2.6.2. Conclusions

Pharmacokinetics

The pharmacokinetics of OAV101B have been evaluated in a limited number of studies which is agreed.

Pharmacodynamics

The mechanism of action (MoA) of OAV101B on the primary pathology of SMA is clear, and has been described adequately. OAV101B is administered intrathecally, but a proportion of the product reaches the systemic circulation and peripheral organs.

Immunological studies of serum antibodies showed that the humoral response to OAV101B is primarily mounted against the capsid. Serum immune response to AAV and SMN protein were recorded, as well as complement activation. None of the investigated immune responses were predictors of efficacy outcomes. However, as the product is administered intrathecally, it is unclear how this data reflects possible immune responses at the site of administration, as immunogenicity in the CSF was not investigated.

Overall, the clinical pharmacology data are acceptable.

5.3. Clinical efficacy

5.3.1. Dose response study

Study CL-102

Study CL-102 was a Phase I/II open-label, dose ranging study to assess efficacy, safety, and tolerability of OAV101B in participants 6 to <60 months of age. The participants were treatment-naive and stratified into 2 age groups at the time of dosing: 6 to <24 months of age, and 24 to <60 months of age, which allow pre-treatment baseline assessment using the Bayley-III for all study patients and the HFMSE for the 24 to <60 months of age group. Eligible patients had a genetic diagnosis consistent with SMA, bi-allelic deletion of SMN1 and 3 copies of SMN2, onset of clinical signs and symptoms consistent with SMA at <12 months of age, and were able to sit, but not stand or walk independently at the time of study entry. These phenotype and genotype characteristics correspond to patients type 2 SMA.

The study enrolled a total of 32 participants into 3 consecutive dose cohorts:

- Dose A is 6.0×10^{13} vg of OAV101B (N=3)
- Dose B is 1.2×10^{14} vg of OAV101B (N=25)
- Dose C is 2.4×10^{14} vg of OAV101B (N=4)

Efficacy was assessed independently for each age cohort, using the HFMSE (for participants 24 months of age and older), and using the gross and fine motor subtests of the Bayley Scales of Infant and Toddler Development version 3 (Bayley-III), a standardized, norm-referenced developmental assessment. Nine items from the Bayley-III GM subtest were selected for inclusion in the Bayley Scales-Motor Milestone Development Survey.

The primary efficacy endpoint by age group was:

- Age group 24 to <60 months: change from baseline in HFMSE
- Age group 6 to <24 months: proportion of patients who achieved the ability to stand without support for at least 3 seconds (Bayley-III GM #40)

The secondary efficacy endpoint for both age groups was the proportion of patients who achieved the ability to walk without assistance, defined as taking at least 5 steps independently displaying coordination and balance (Bayley-III GM #43).

For the age group 6 to <24 months, the results of exploratory efficacy analyses evaluating motor function provide evidence of efficacy in this younger population:

- Change from baseline in Bayley-III GM and FM total scores

Change in HFMSE for patients who continued in the study past 24 months of age.

Summary of results in participants 24 to <60 months of age

Of the 32 participants enrolled into CL-102, 12 participants were aged 24 to <60 months, and all received the therapeutic dose of OAV101B $1.2x10^{14}$ vg (Dose B).

At Month 12, the LSM change from baseline in HFMSE total score was 6.0 (95% CI: 3.7 to 8.3), and 11 of the 12 participants (91.7%) had an increase of at least 3 points in HFMSE total score (Table 6 below).

Table 6: HFMSE change from baseline at month 12 in the 24 to <60 months age group in study CL-102 (ITT Set)

Analysis	Statistic	Cohort 2 OAV101B 1.2x10^{14} vg N=12
HFMSE change from baseline		
Baseline	n	12
	Mean (SD)	14.8 (9.98)
	Median (Min, Max)	12.0 (3, 32)
Month 12: actual value	n	12
	Mean (SD)	21.3 (11.94)
	Median (Min, Max)	16.5 (6, 40)
Month 12: change from baseline	n	12
	Mean (SD)	6.6 (7.34)
	LS Mean (95% CI)	6.0 (3.7, 8.3)
Proportion of participants achieving ≥3-point increase in HFMSE	Yes, n (%)	11 (91.7)
	No, n (%)	1 (8.3)

Source: SCE-Table 2-10

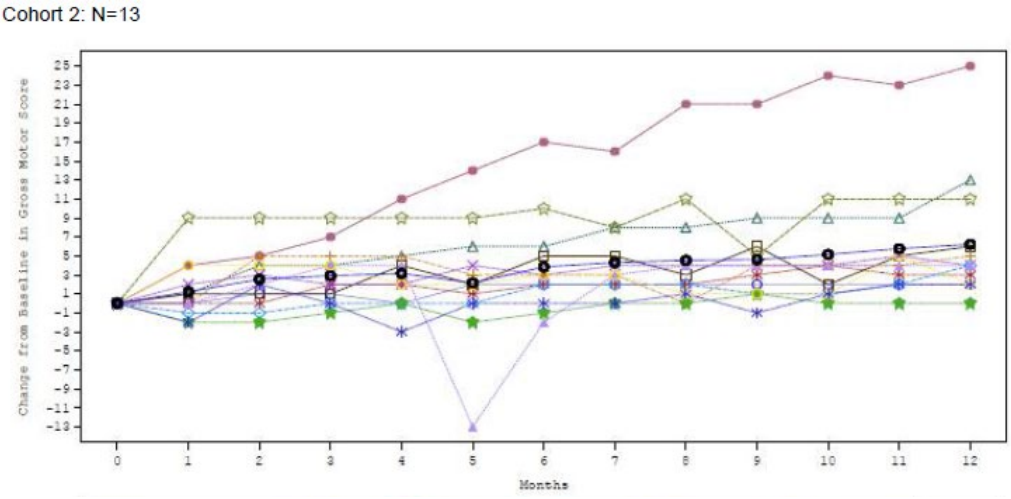
Summary of results for participants 6 to < 24 months of age

Of the 32 participants enrolled in the study, 13 were 6 to <24 months of age and received the proposed therapeutic dose of 1.2×10^{14} vg (Dose B), which was selected for the subsequent Phase III OAV101B studies. Another three participants in this age group received Dose A (6.0×10^{13} vg), and 4 participants received Dose C (2.4×10^{14} vg).

The proportion of patients who achieved the primary endpoint stand alone (Bayley-III GM #40) was 7.7% (1/13 participants) (Study CL-102-Section 11.1.1), with the same individual achieving both endpoints. One additional patient in this age range, who received the lower dose of $6.0\text{x}10^{13}$ vg achieved the motor milestone stands alone.

Analysis of changes in Bayley-III GM and FM subtest scores showed improvements in both gross and fine motor skills during the follow-up period of 12 months. The mean (SD) change from baseline at Month 12 was 6.2 (6.71) for the Bayley-III GM Subtest total score (displayed in figure below), and 11.4 (5.41) for the Bayley FM Subtest total score (Study CL-102-Table 14.2.4.3).

Figure 1: Spaghetti plot of change from baseline in Bayley-III GM subtests total score at each post-baseline visit up to 12 months in patients 6 to <24 months of age at time of dosing



Six of the 13 participants demonstrated new developmental milestones, including one patient who achieved both the primary and the secondary endpoint. The remaining 7 patients maintained the milestone sitting without support from baseline and during the study.

Eleven of the 13 participants reached 24 months of age during the study. Of these 11 patients, 6 had at least 7 months duration of post-baseline HFMSE assessment.

The mean (SD) score at the first HFMSE assessment was 10.5 (3.73) (range 6 to 15), and the mean (SD) change from initial administration (when patients reach 24 months of age) to 7 months post-baseline was 6.7 (4.72) (range 1 to 14).

5.3.2. Justification for the choice of dose

The proposed OAV101B dosage regimen for the treatment of SMA is $1.2x10^{14}$ vg administered as a one-time IT injection, which was selected for the Phase III studies B12301 and B12302. It was based on the totality of clinical and nonclinical data with the following considerations (SCP-Section 3.4.2).

The selection of doses for investigation in the Phase I/II dose-ranging Study CL-102 was informed by nonclinical pharmacology studies. In nonclinical studies, transduction and expression of SMN in >40% of lumbar spinal cord motor neurons was effective at improving SMA-like disease phenotypes in a mouse model of SMA. In neonatal mice, OAV101 mRNA was expressed in the spinal cord at 24 weeks post dose when dosed neonatally via CSF delivery, indicating sustained transgene expression. In NHPs, an IT dose of ~$1.2x10^{13}$ vg achieved transduction of >55% spinal cord motor neurons, exceeding 40% required for efficacy [Nonclinical Overview]. This NHP dose was scaled 10-fold to the human dose based on approximately 10-fold difference in CSF volume between monkeys and humans (Sullivan et al 2020, Matsuzawa et al 2001). These data support that OAV101 delivered IT at a clinical dose of $1.2x10^{14}$ vg will effectively transduce target tissues in the CNS and produce sustained SMN expression and desired efficacy in SMA patients. The clinical dose of $1.2x10^{14}$ vg was selected as the middle dose in Study CL-102. On the basis of both nonclinical data and clinical data from Study CL-102, this dose was evaluated in the pivotal Study B12301.

The IT route of administration delivers OAV101 locally into the CSF, where it acts directly on the spinal cord as the primary site of action. Given that the CSF volume is approximately constant (~150 mL) in humans aged 2 years and above (Matsuzawa et al 2001, Mandell et al 2015), a fixed dose will result in

similar OAV101 concentrations in the CSF of patients regardless of age or body weight. The CSF volume of children aged 6 months is approximately 20% lower (120 vs 150 mL) compared to those 2 years and above (Matsuzawa et al 2001), thus the same fixed dose of OAV101B (1.2x1014 vg) is predicted to result in CSF OAV101 concentrations approximately 20% higher in patients aged 6 months to <2 years, as claimed by the Applicant. Nonclinical data in NHPs showed that OAV101 biodistribution to the spinal cord plateaued at the lowest dose tested (1.2x1013 vg, human equivalent dose 1.2x1014 vg), suggesting that higher OAV101 concentrations in the CSF will not lead to additional spinal cord transduction [Nonclinical Overview-Section 3.3]. Given that the 1.2x1014 vg fixed dose of OAV101B was safe and well tolerated in patients aged 6 to <60 months in Study CL-102, the same fixed dose was recommended by the Applicant in patients of this age range. Dose administration based upon a scaling factor, e.g., body weight, is unnecessary.

The selection of the $1.2x10^{14}$ vg clinical dose is also supported by nonclinical toxicology studies. In NHPs, microscopic findings in the CNS and peripheral nervous system consisting of mononuclear cell inflammation, neuronal degeneration, increased satellite glial cells/neuronal loss, axonal degeneration, and/or gliosis were observed at a dose of 1.2×10^{13} vg (human equivalent dose of $1.2x10^{14}$ vg). These CNS and peripheral nervous system findings at the $1.2x10^{13}$ vg dose were considered nonprogressive, and resolved or resolving at 52-weeks post dose. More severe and unresolved neurotoxicity were observed in animals treated with higher dose levels ($3x10^{13}$ and $6x10^{13}$ vg/animal) at 52-week post dose [Toxicology Written Summary]. The $1.2x10^{13}$ vg dose in NHPs achieved similar OAV101 distribution and persistence of the transgene at site of action (i.e. spinal cord) compared to the higher dose levels [Pharmacokinetics Written Summary-Section 9]. Thus, these data suggest that the $1.2x10^{14}$ vg clinical dose will achieve maximal spinal cord transduction, and thus efficacy, while minimizing the potential risk for neurotoxicity. At this dose level, OAV101B biodistribution and expression in most peripheral tissues such as liver were lower than Zolgensma, suggesting a reduced potential for associated adverse events.

The effect of age on OAV101B biodistribution or transgene expression at the site of action was not assessed in nonclinical species or human. However, published nonclinical data in cats suggests that AAV9 vector transduction in motor neurons via CSF delivery was not affected by age (Bucher et al 2014). Importantly, OAV101B was efficacious, safe, and well tolerated in the target patient population, as claimed by the Applicant.

In summary, the OAV101 $1.2x10^{14}$ vg dose via IT route of administration was selected based on the totality of nonclinical and clinical data, taking into consideration effective motor neuron transduction and persistence of vector at the site of action, minimizing potential risk of neurotoxicity, and a reduced potential for AEs such as hepatotoxicity. The selection of this dose also considered the approximately 10-fold difference in CSF volume between NHPs and humans. This dose demonstrated clear efficacy and was safe and well tolerated in the clinical studies.

5.3.3. Main study

5.3.3.1. Study B12301

5.3.3.1.1. Study title

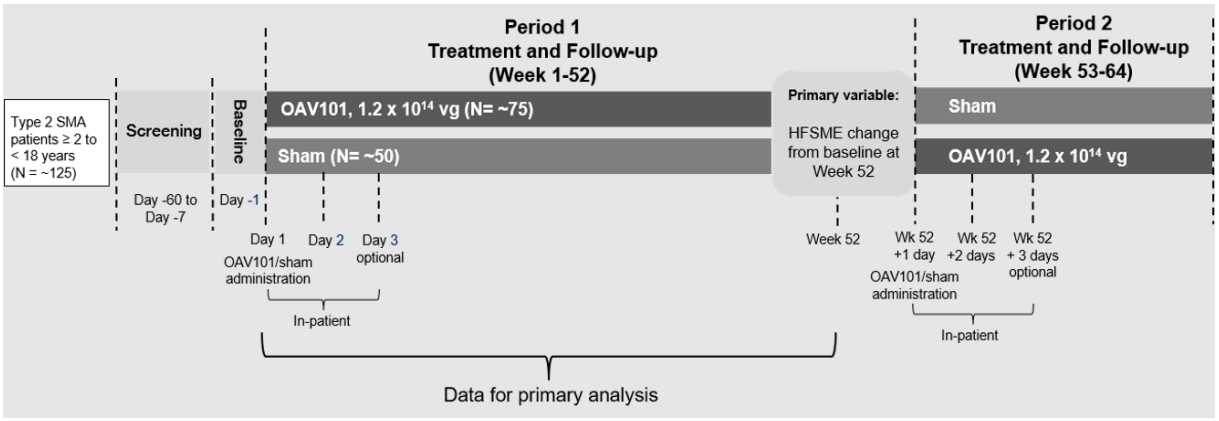
Study B12301: A randomized, sham-controlled, double-blind study to evaluate the efficacy and safety of intrathecal (IT) OAV101 in patients with later onset Type 2 spinal muscular atrophy (SMA) who are ≥ 2 to < 18 years of age, treatment naive, sitting, and never ambulatory

5.3.3.1.2. Study design

Study B12301 is a Phase III multi-center, single dose ($1.2x10^{14}$ vg), randomized, sham controlled, double-blind study that investigates the efficacy, safety and tolerability of OAV101B in treatment naive, sitting and never ambulatory SMA patients 2 to <18 years of age.

The study comprised two periods: a 52-week Period 1, and a 12-week Period 2 (i.e. up to Week 64). In Period 1, participants were randomized 3:2 to receive OAV101B or sham administration and were followed up to Week 52. Thereafter, eligible participants entered into Period 2, where who received a sham procedure in Period 1 received OAV101B, and vice-versa. Period 1 was the basis for the primary analysis.

Figure 2: Study schema



Patient population

The study population comprised male and female SMA patients 2 to <18 years of age. Eligible patients were: treatment naïve for prior SMA-targeted treatment; genetically confirmed biallelic SMN1 pathogenic variants; onset at ≥6 months of age, and able to sit independently but never walked independently. Eligible patients were symptomatic, and able to perform motor function assessments, with no lower limit for HFMSE and RULM scores (Study B12301 Protocol-Section 5).

The study enrolled participants from 14 countries, including Europe (Denmark), Asia (Vietnam, China, India, Malaysia, Singapore), South Africa, Saudi Arabia, Mexico, Brazil, and USA (Study B12301-Table 14.1-2.1.4).

Main inclusion criteria:

- Diagnostic confirmation during screening period of 5q SMA
- Treatment naive (historical or current use) for SMN-targeting therapies (e.g., risdiplam, nusinersen)
- ≥2 years and < 18 years of age at screening visit 1
- Onset of clinical signs and symptoms at ≥6 months of age
- Complete HFMSE assessment, with an available total score at screening
- Able to sit independently at screening, but has never had the ability to walk independently

Main exclusion criteria:

- Anti-AAV9 antibody titer reported as elevated (reference to > 1:50 or a validated result consistent with being elevated) at screening as determined by ligand binding immunoassay*.
- Infections

- Hepatic dysfunction
- Invasive / noninvasive ventilation
- Complications at screening that would interfere with motor efficacy assessments including:
  - Severe contractures (e.g., hip flexion contracture) which limits the participant's ability to be positioned in prone for HFMSE item administration
  - Scoliosis with a Cobb angle > 40 degrees evident on x ray examination at Screening
- Surgery for scoliosis or hip fixation in the 12 m
- Clinically significant abnormalities during screening period and/or during baseline period as determined by the investigator. Specifically, patients with clinically significant sensory abnormalities, or those who were unable to obtain Sensory Nerve Action Potential (SNAP), were excluded.

*The three anti-AAV9 assays used to assess eligibility in the study provided a consistent approach to the exclusion criteria, i.e. anti-AAV9 antibody titer >1:50 or a validated result consistent with being elevated.

Randomisation and blinding

Eligible participants were randomized at Screening Visit 3 via an Interactive Response Technology (IRT) to one of the treatment arms in a 3:2 ratio (Study B12301 Protocol-Section 6.4).

With the exception of the designated unblinded personnel at the sites and the Sponsor, all other personnel remained blinded to the identity and sequence of the treatment assignments from the time of randomization until database lock (Study B12301 Protocol-Section 6.5).

OAV101B was supplied to the unblinded study-site pharmacist or equivalent per local practice. The pharmacist (or equivalently trained individual per local practice) prepared the appropriate dosage. An unblinded interventional radiologist or qualified specialist (e.g., neurologist) administered OAV101B and performed the sham procedure. Strict blinding was maintained once the participant left the procedure room.

Emergency code breaks could be undertaken when required to treat the participant safely, and were performed using the IRT (Study B12301 Protocol-Section 6.7.3).

Treatment

OAV101B was administered by lumbar IT injection as a single dose of $1.2x10^{14}$ vector genomes under sedation/anesthesia. Participants were placed in the Trendelenburg position, tilted head-down at 30° for 15 minutes following administration of OAV101 to enhance distribution to cervical and brain regions.

Participants randomized to the control arm received a sham procedure, consisting of a needle prick that broke the skin, but without performing lumbar puncture.

Study treatment (OAV101B or sham procedure) was administered under sterile conditions in an ICU patient room or other appropriate setting by the designated unblinded physician (Study B12301 Protocol-Section 6.3).

Participants randomized to OAV101B arm received immunomodulatory therapy with prednisolone, at the regime shown in Table 7 below.

Participants randomized to the sham procedure arm received matching placebo (as claimed by the Applicant) by the unblinded pharmacist.

Table 7: Prednisolone prophylaxis and placebo administration

Period	Dosing schedule	Dose
Pre-injection	Approximately 24 hours prior to OAV101B injection/sham procedure	Prednisolone or placebo orally 1 mg/kg/day, up to a maximum of 60 mg/day
Post-injection	30 days (including the day of OAV101B administration/sham procedure)	Prednisolone or placebo orally 1 mg/kg/day, up to a maximum of 60 mg/day
Tapering	at least 28 days	Decrease dose by 0.20 mg/kg per week Note: Dose should be tapered down to ≤ 5 mg/day before stopping

The use of other immunosuppressive therapies was prohibited in the study.

Study assessments

Efficacy was assessed using the HFMSE and RULM (respectively primary and secondary efficacy endpoints).

HFMSE is a scale specifically designed to assess motor function changes in children with SMA to give objective information on motor ability and clinical progression and is commonly used in clinical trials, natural history studies, and clinical practice (Glanzman et al 2011). The HFMSE consists of a series of 33 motor tasks that assess different aspects of motor performance. Each item is scored on a 3-point Likert scale from 0 (no response) to 2 (full response), with a total score range of 0 to 66. A higher score indicates a higher ability level.

RULM is a validated, SMA-specific assessment that measures motor performance in the upper limbs from childhood through adulthood in ambulatory and never ambulatory individuals with SMA, and weaker individuals who have a floor effect or very low score on the Hammersmith Functional Motor Scale (HFMS) (Mazzone et al 2017). The revised version of the test consists of 19 scorable items: 18 items scored on a 0 (unable) to 2 (full achievement) scale, and one item that is scored from 0 (unable) to 1 (able). These item scores are summed to give a total score ranging from 0 to 37 points with higher scores reflecting better ability. The RULM consists of upper limb performance items that are reflective of reachable space and activities of daily living (i.e., raise a can to mouth as if drinking, take a coin and place it in a box, and remove the lid of a container). RULM has been used in clinical trials assessing the efficacy of SMN-targeting therapy (Mercuri et al 2018).

The HFMSE and RULM were administered by qualified clinical evaluators at Screening 1 and 2, Baseline, Week 4, and every 4 weeks thereafter until Week 52. Rescheduling of the assessment administration was recommended when any factor significantly impeded the participant's ability to perform the motor assessment.

5.3.3.1.3. Objectives and estimands

Primary objective

The primary objective of the study was to compare the efficacy of OAV101B vs. sham control as measured by the change from baseline in HFMSE total score. The primary endpoint was the change from baseline in HFMSE total score at the end of Follow-up Period 1 in the overall study population (2 to < 18 years age group).

The aim was to estimate the treatment effect of OAV101B compared to the sham procedure, for the target population on the primary endpoint.

The statistical hypothesis for the primary endpoint being tested was that there is no difference in the change from Baseline in HFMSE total score at the end of Follow-up Period 1 in the OAV101B group compared to the sham group.

Let pj denote the change from Baseline in HFMSE total score at the end of Follow-up Period 1 for treatments j, j=0, 1 where:

- 0 corresponds to sham procedure
- 1 corresponds to OAV101

In statistical terms, H0: p1=p0, HA: p1≠p0, i.e.:

- H0: OAV101 is not different from sham procedure with respect to the change from Baseline in HFMSE total score at the end of Follow-up Period 1.
- HA: OAV101 is different from sham procedure with respect to the change from Baseline in HFMSE total score at the end of Follow-up Period 1.

Estimands for the primary objective

Table 8: Estimands for primary objective

Population	**Treatment naïve patients with SMA aged 2 to <18 years, who were able to sit but had never walked independently.**
Treatment conditions	Assignment to OAV101B, regardless of use of prohibited concomitant medications not for the intent to treat SMA, compared to assignment to sham procedure, regardless of use of prohibited concomitant medications not for the intent to treat SMA.
Endpoint (variable)	Change from baseline to the end of Follow-up Period 1 in HFMSE total score.
Population-level summary	Difference between treatment groups in LSM change from baseline in HFMSE total score up to the end of Follow-up Period 1
Intercurrent events and strategy to handle them	
Study discontinuation due to reasons other than death	Hypothetical. It is assumed that participants discontinuing the study prior to Week 52 would follow the same trend as participants who continued and remained in the study for the full 52 weeks. Data collected up to the point of discontinuation will be included in the MMRM.
Use of prohibited concomitant medications not for the intent to treat SMA	Treatment policy. Assessments collected while/after receiving prohibited concomitant medications will be included in the analyses
Use of prohibited concomitant medications for the intent to treat SMA (i.e., nusinersen, risdiplam)	Hypothetical. Assessments collected while/after receiving prohibited concomitant medications will not be included in the analyses. Only data collected up to the point of initiating a prohibited medication for the intent to treat SMA will be included in the MMRM and the data collected after the initiation of prohibited medication for the intent to treat SMA will be considered as missing.

Population	Treatment naïve patients with SMA aged 2 to <18 years, who were able to sit but had never walked independently.
Study discontinuation due to death	Hypothetical: The worst score for HFMSE will be imputed for participants who discontinue the study due to death, and this imputed score will be utilized in the analysis. The "worst score" refers to the worst (lowest) HFMSE score collected for the participant who had the death event throughout the trial (considering both the baseline and post-baseline period). It is anticipated that this intercurrent event is unlikely to occur during this study.

The primary clinical question of interest is: What is the effect of OAV101B treatment versus the sham procedure on change from baseline in HFMSE total score after treatment in sitting but never ambulatory patients aged 2 to < 18 years with Type 2 SMA, regardless of study discontinuation or receipt of prohibited concomitant medications not for the intent to treat SMA?

The justification for the primary estimand is that it will capture both the effect of OAV101B and the effect of additional medications not for the intent to treat SMA, mirroring the conditions in clinical practice.

Statistical methods for estimation and sensitivity analysis on primary estimands

The primary efficacy endpoint variable was analysed using a linear mixed effects repeated measures model (MMRM) with the observed change from Baseline in HFMSE total score at all post-Baseline visits (through the end of Follow-up Period 1) as the dependent variable. The fixed effects in the MMRM model included treatment, strata, scheduled visit, treatment by visit interaction, and baseline HFMSE total score as covariate. An unstructured covariance matrix was used.

To account for variability in the completion of motor assessments (HFMSE or RULM) occurring at Week 52 visit that could impact the MMRM assessment, the motor assessment score at Week 48 and Week 52 were averaged for the purpose of creating the end of Follow-up Period 1 timepoint. If just one (and not both) of the assessments collected at these two visits has a total score available, then the end of Follow-up Period 1 assessment was defined as the single assessment total score collected at Week 48 or Week 52.

The null hypothesis was rejected if the two-sided p-value for the LSM difference between the OAV101B arm and the sham arm at the end of Follow-up Period 1 was less than the alpha level dictated by the pre-specified multiple testing procedure (Study B12301 SAP-Section 2.5.2).

The primary efficacy analysis was conducted using the FAS.

To assess the potential impact of excluding participants from the primary analysis who were randomized into the study but did not receive treatment, the primary efficacy analysis was repeated using the ITT Set.

Secondary objectives

The secondary objectives and endpoints are listed in Table 9 below. The statistical hypothesis for the secondary endpoints was the same as for the primary endpoint, i.e. that there is no difference in the change from Baseline in HFMSE total score at the end of Follow-up Period 1 in the OAV101B group compared to the sham group.

Table 9: Secondary efficacy objectives and related endpoints for study B12301

Secondary efficacy objective	Endpoints for secondary objective
To compare the efficacy of OAV101 IT vs. sham control in two patient age groups: 2 to < 5 years (HFMSE, RULM); 2 to < 18 years (RULM)	• Change from baseline in HFMSE total score at the end of Follow-up Period 1 in the 2 to < 5 years age group • Achievement of at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1 in the overall study population • Achievement of at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1 in the 2 to < 5 years age group • Change from baseline in RULM at the end of Follow-up Period 1 in the 2 to < 18 years age group • Change from baseline in the RULM at the end of Follow-up Period 1 in the 2 to < 5 years age group

The exploratory efficacy objectives are listed in the table below. No hypothesis testing was performed for exploratory endpoints.

Estimands for the secondary objectives

The secondary estimand for the continuous secondary efficacy endpoints is defined similarly to the primary estimand (Table 15).

The secondary estimand for the dichotomous secondary efficacy endpoint after treatment (i.e., achievement of at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1) is described by the attributes listed in Table 10 below.

Table 10: Estimands for secondary objective, dichotomous secondary efficacy endpoint

Population	Patients with SMA aged 2 to <18 years who were able to sit but had never walked independently).
Treatment conditions	Assignment to OAV101B, regardless of use of prohibited concomitant medications not for the intent to treat SMA, compared to assignment to sham procedure, regardless of use of prohibited concomitant medications not for the intent to treat SMA.
Endpoint (variable)	Proportion of participants achieving at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1.
Population-level summary	Odds ratio between treatment arms for the proportion of participants achieving at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1.
Intercurrent events and strategy to handle them	
Study discontinuation due to reasons other than death	Hypothetical. It is assumed that participants discontinuing the study prior to Week 52 would follow the same trend as participants who continued and remained in the study for the full 52 weeks. Data collected up to the point of discontinuation were included in the Generalized Linear Mixed Effects Model.
Use of prohibited concomitant medications not for the intent to treat SMA	Treatment policy. Assessments collected while/after receiving prohibited concomitant medications will be included in the analyses.
Use of prohibited concomitant	Hypothetical. Assessments collected while/after receiving prohibited concomitant medications were not included in the analyses. Only data collected up to the point

Population	Patients with SMA aged 2 to <18 years who were able to sit but had never walked independently).
medications for the intent to treat SMA (i.e., nusinersen, risdiplam)	of initiating a prohibited medication for the intent to treat SMA was included in the Generalized Linear Mixed Effects Model and the data collected after the initiation of prohibited medication for the intent to treat SMA was considered as missing.
Study discontinuation due to death	Hypothetical. The worst score for HFMSE was planned to be imputed for participants who discontinued the study due to death, and this imputed score was to be utilized in the analysis. The "worst score" refers to the worst (lowest) HFMSE score collected for the participant who had the death event throughout the study (considering both the baseline and post-baseline period). It was anticipated that this intercurrent event was unlikely to occur during this study.

Statistical methods for estimation and sensitivity analysis on the secondary estimands

The continuous secondary endpoints of change from baseline in HFMSE and RULM total scores in the 2 to <5 years age group or the whole study population were analyzed in a similar manner to how the primary endpoint was analyzed using a MMRM. Line plots were produced for each of the continuous secondary endpoints to display the LSMs for each treatment arm and associated 95% CIs at each visit through the end of Follow-up Period 1.

The dichotomous endpoint of the proportion of responders (defined as participants who achieved at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1) in the overall study population was analyzed by a logistic regression model with firth correction including treatment, strata and the baseline HFMSE total score as covariates. For the 2 to <5 years age group, the proportion of responders was analyzed by a generalized linear mixed effects model including treatment, visit, treatment by visit interaction, strata and the baseline HFMSE total score as covariates. A compound symmetry matrix was used.

In all cases, estimates (difference or odds ratio) of OAV101B arm compared to the Sham arm with associated 95% CIs and two-sided p-values were provided.

The same methodology for sensitivity of the primary endpoint were applied to the secondary endpoints.

Additionally, another sensitivity analysis will be performed for the RULM secondary endpoints. The RULM has been validated in SMA patients 30 months of age to adults. Therefore, subjects whose age at screening visit 1 was < 30 months of age will be excluded from this analysis

Testing procedure

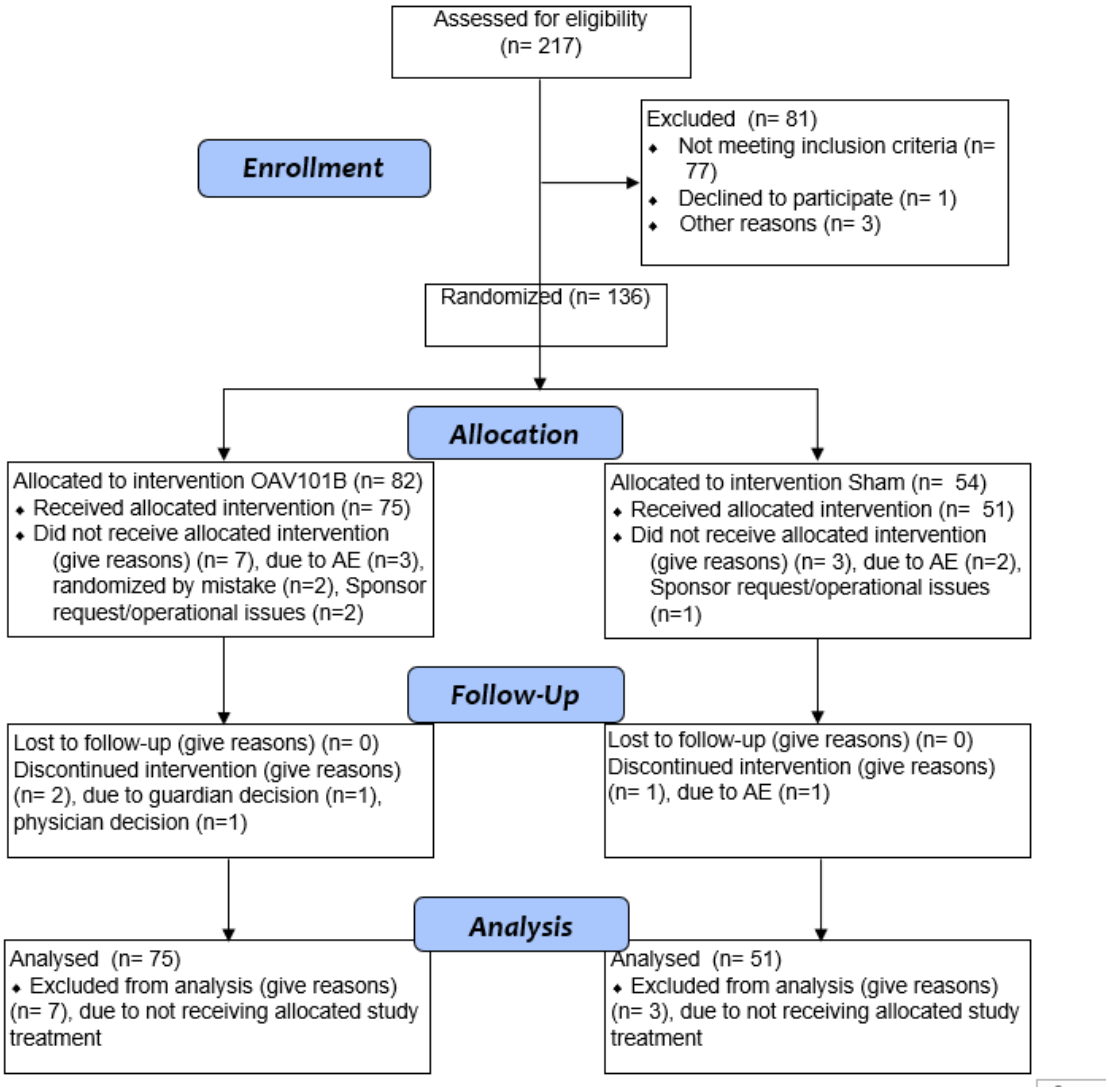
A hierarchical gate-keeping procedure and the Hochberg method was used to maintain the overall 2-sided study-wise type I error rate at 0.05 for the primary endpoint and secondary efficacy endpoints which are positively correlated. The primary endpoint, the change from baseline in HFMSE total score to the end of Follow-up Period 1 in the OAV101B arm compared with the Sham-control arm was initially tested at a 2-sided significance level of 0.01 and 0.04, for the overall study population (2 to <18 years old) and the younger age group (2 to <5 years old), respectively. If the primary endpoint and both secondary endpoints were statistically significant for either age group (2 to <5 years subgroup or 2 to <18 years overall group), the corresponding significance level was to be carried over to the primary endpoint for the other age group, and the primary endpoint was to be re-compared to the Sham-control arm at a 2-sided significance level of 0.05 (full alpha). If the primary endpoint of one age group was statistically significant but not at the secondary endpoints level, then only half of the initial alpha of the age group could be transferred to the primary endpoint of the other age group.

5.3.3.1.4. Results

Participant flow and numbers analysed

The first participant was enrolled (randomized) on 14-Mar-2022 and treated on 24-Mar-2022. The last participant was treated on 22-Nov-2023 (Study B12301-Section 10.1).

Figure 3: Participant flow



217 participants were screened.
81 participants did not complete the screening: 77 did not meet inclusion/exclusion criteria, due to elevated anti-AAV9 titer (n=27), infections (n=14), and severe contractures or scoliosis (n=14). Screen failure in the remaining 4/81 participants was due to guardian decision (n=1), physician decision (n=2), and (n=1) (Study B12301-Listing 16.2.1-2).
136 participants were randomized (OAV101B n=82, Sham n=54), and were included in the ITT.
10/136 participants were randomized but not treated (OAV101B n=7, Sham n=3), of which n=5 due to AEs.
126 participants were treated (OAV101B n=75, Sham n=51), and included in the FAS.
123 participants completed Period 1 (Week 52).

Intercurrent events

- Study discontinuation due to reasons other than death: OAV101B n=2/75, Sham n=1/51. Data collected up to the point of discontinuation were included in the MMRM.
- Use of prohibited concomitant medications did not occur.
- Study discontinuation due to death did not occur.

Deviations from study plan

The study protocol was amended 4 times. All amendments were released after the First Patient First Visit (FPFV) of 01-Feb-2022, and prior to the data cut-off date of 12-Nov-2024 (LPLV at Week 52). Main protocol changes are outlined in table 11 below.

Table 11: Protocol amendments

Version and date	Summary of key changes
Amendment 4 (17-May-2024)	Text updated to enhance consultation between the Principal Investigator and Sponsors for patients retesting of abnormal laboratory and other clinical parameters, to clarify interpretation of total score, and to ensure compliance with expedited reporting of potential Hy's Law cases based on health authority guidance.
Amendment 3 (02-Jun-2023)	Several exclusion criteria were revised so that they are now anchored to the Day 1 visit (day of OAV101B administration or the sham procedure). Finally, a theoretical risk of tumorigenicity due to vector DNA integration has been added to the OAV101 risks section.
Amendment 2 (08-Aug-2022)	The Central Treatment Site (hybrid model) was removed, which had not been utilized and was therefore not applicable for study execution. Adaptations were made to clarify that the threshold used for anti-AAV9 antibody titer is one that was reported to be elevated. Due to the nature of the immunoassay used to evaluate anti-AAV9 antibody titer, the reported titer value as elevated may be different. In addition, further adaptations were made for clarity, including mirroring exclusion criteria in Treatment Period 2 from Treatment Period 1 for safety considerations, and updating the section discussing OAV101 risks.The visit schedule for troponin I collection was modified to better align with timepoints for electrocardiogram and echocardiogram assessments. The revised visit schedule for troponin I now aligns with ECG and ECHO, as well as with other ongoing OAV101 studies. Sensory nerve action potential (SNAP) evaluation on asymptomatic participants was no longer applicable and therefore was removed for clarity. All patients underwent a neurological exam and SNAP at screening, and those with clinically significant sensory abnormalities in the neurological examination or those who were unable to obtain SNAP, were not eligible. Post-treatment, SNAP was ONLY performed if there were sensory abnormalities in the neurological examination. Therefore, there were no cases of asymptomatic participants for SNAP evaluation.
Amendment 1 (11-Mar-2022)	The data analysis and statistical methods section were revised to include the following two secondary endpoints: • Achievement of at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1 in the overall study population • Achievement of at least a 3-point improvement from baseline in HFMSE total score at the end of Follow-up Period 1 in the 2 to <5 years age group

Version and date	Summary of key changes
	The schedule of SNAP assessments were modified in view of ethical consideration and clinical best practice to ensure evaluation and monitoring of potential neurotoxicity SNAPs were evaluated in all patients at screening as described in the previous protocol version. However SNAP evaluation at five post-treatment visits was adjusted. The neurological examination, which was already incorporated in the protocol at every post-treatment visit, was used to primarily evaluate potential sensory abnormalities in patients. Upon detection of a potential sensory abnormality, a SNAP assessment would then be performed as a secondary evaluation. Edits were made to the inclusion/exclusion criteria to provide additional clarity. Inclusion criterion #2 related to 2-4 copies of SMN2 in patients with SMA Type 2 has been updated due to limitations with the SMN2 assay, which cannot distinguish between 4 and greater than 4 copy numbers. SMN2 copy number is not critical to defining the study population of Type 2 SMA. The study population can be defined by genetic confirmation of 5q SMA along with clinical symptoms of being able to sit independently but never having walked independently (inclusion criterion #7). In addition, several exclusion criteria were added with rationale provided in the Summary of key changes table. Editorial changes as well as changes to align with Novartis' Clinical Trial Protocol Template Version 5.0 (14-Jan-2022) were made throughout for clarity.
Original protocol v0 (23-Jul-2021)	

Baseline data

Demographics

Demographic information of study B12301 is provided in Table 12 below.

Table 12: Demographics by treatment arm at period 1 (FAS)

Characteristic Categories/Statistics	OAV101B N=75	Sham N=51	Overall N=126
Age (years) at screening visit 1			
N	75	51	126
Mean (SD)	5.71 (3.575)	5.68 (3.045)	5.70 (3.358)
Median	4.50	4.67	4.54
Min-Max	2.0 - 16.5	2.0 - 14.1	2.0 - 16.5
Age (years) at dosing on Day 1			
N	75	51	126
Mean (SD)	5.89 (3.578)	5.87 (3.047)	5.88 (3.360)
Median	4.67	4.82	4.69
Min-Max	2.1 - 16.6	2.4 - 14.2	2.1 - 16.6
Sex - n (%)			
Male	34 (45.3)	28 (54.9)	62 (49.2)
Female	41 (54.7)	23 (45.1)	64 (50.8)
Race - n (%)			
White	7 (9.3)	7 (13.7)	14 (11.1)
Black or African American	5 (6.7)	4 (7.8)	9 (7.1)
Asian	49 (65.3)	25 (49.0)	74 (58.7)
Native Hawaiian or Other Pacific Islander	0	0	0
American Indian or Alaska Native	2 (2.7)	5 (9.8)	7 (5.6)
Multiple	0	0	0

Characteristic Categories/Statistics	OAV101B N=75	Sham N=51	Overall N=126
Unknown	12 (16.0)	10 (19.6)	22 (17.5)
Ethnicity - n (%)			
Hispanic or Latino	6 (8.0)	9 (17.6)	15 (11.9)
Not Hispanic or Latino	48 (64.0)	29 (56.9)	77 (61.1)
Not reported	1 (1.3)	1 (2.0)	2 (1.6)
Unknown	8 (10.7)	2 (3.9)	10 (7.9)
Missing	12 (16.0)	10 (19.6)	22 (17.5)

Percentages are calculated based on the number of participants in the Full analysis set (N).

Age (years) at screening visit 1 = [(recorded years*12) + (recorded months)] / 12.

Age (years) at dosing on day 1 = [Age (days) at screening visit 1 + (Day 1 visit date - Screening 1 visit date)] / 365.25.

Source: Study B12301-Table 14.1-2.1.4

Baseline disease characteristics

Baseline disease characteristics are summarized in Table 13 below.

Table 13: Baseline characteristics and SMA medical history by treatment at period 1 (FAS)

Characteristic Categories/Statistics	OAV101B N=75	Sham N=51	Overall N=126
Randomization strata assignment - n (%)			
Age 2 to <5, pre-treatment HFMSE score ≤15	16 (21.3)	10 (19.6)	26 (20.6)
Age 2 to <5, pre-treatment HFMSE score >15	26 (34.7)	19 (37.3)	45 (35.7)
Age 5 to <13, pre-treatment HFMSE score ≤10	7 (9.3)	5 (9.8)	12 (9.5)
Age 5 to <13, pre-treatment HFMSE score >10	21 (28.0)	15 (29.4)	36 (28.6)
Age 13 to <18, pre-treatment HFMSE score is not considered	5 (6.7)	2 (3.9)	7 (5.6)
Highest motor function achieved - n (%) *, **			
Non-sitting	0	0	0
Sitting	41 (54.7)	25 (49.0)	66 (52.4)
Stands with assistance	17 (22.7)	16 (31.4)	33 (26.2)
Stands independently	2 (2.7)	1 (2.0)	3 (2.4)
Walks with assistance	15 (20.0)	9 (17.6)	24 (19.0)
Walks independently	0	0	0
Number of participants with hospitalizations for pneumonia with respiratory failure - n (%)	10 (13.3)	8 (15.7)	18 (14.3)
Number of hospitalizations for pneumonia with respiratory failures			
N	75	51	126
Mean (SD)	0.2 (0.80)	0.3 (0.93)	0.3 (0.85)
Median	0.0	0.0	0.0
Min-Max	0 - 6	0 - 4	0 - 6
Number of participants with hospitalizations for pneumonia without respiratory failure - n (%)	18 (24.0)	14 (27.5)	32 (25.4)
Number of hospitalizations for pneumonia without respiratory failure			
N	75	51	126
Mean (SD)	0.4 (0.84)	0.8 (1.83)	0.6 (1.34)

Characteristic Categories/Statistics	OAV101B N=75	Sham N=51	Overall N=126
Median	0.0	0.0	0.0
Min-Max	0 - 4	0 - 8	0 - 8
Total time observed sitting independently at screening (seconds)			
N	75	51	126
Mean (SD)	42.9 (21.85)	37.2 (21.30)	40.6 (21.73)
Median	60.0	30.0	57.0
Min-Max	10 - 60	10 - 60	10 - 60
Age (months) at SMA symptom onset**			
N	75	51	126
Mean (SD)	12.7 (5.81)	12.6 (5.48)	12.7 (5.66)
Median	11.0	12.0	11.0
Min-Max	6 - 31	6 - 33	6 - 33
SMA Symptoms - n (%)			
Hypotonia	70 (93.3)	49 (96.1)	119 (94.4)
Limb Weakness	72 (96.0)	47 (92.2)	119 (94.4)
Pneumonia	26 (34.7)	18 (35.3)	44 (34.9)
Other Respiratory Symptoms	4 (5.3)	10 (19.6)	14 (11.1)
Constipation	15 (20.0)	19 (37.3)	34 (27.0)
Swallowing or Feeding Difficulties	2 (2.7)	2 (3.9)	4 (3.2)
Tongue Fasciculations	40 (53.3)	26 (51.0)	66 (52.4)
Developmental Delay	72 (96.0)	50 (98.0)	122 (96.8)
Other	2 (2.7)	1 (2.0)	3 (2.4)

* Based on highest motor milestone that the child ever achieved, and may not be the baseline motor milestone.

** Based on caregiver reports.

Percentages are calculated based on the number of participants in the Full analysis set (N).

Age (years) at screening visit 1 = [(recorded years*12) + (recorded months)] / 12.

Age (years) at dosing on day 1 = [Age (days) at screening visit 1 + (Day 1 visit date - Screening 1 visit date)] / 365.25.

Source: Study B12301-Table 14.1-2.1.4

Mean age at symptom onset, as based on caregiver's reports, was 12.7 months (range 6 to 33 months), and the most frequent symptoms were developmental delay (96.8%), hypotonia (94.4%), limb weakness (94.4%), and tongue fasciculations (52.4%).

None of the participants received prior SMN-targeted treatment.

The highest motor function achieved prior to enrolment was sitting in 52.4% of participants, stands with assistance in 26.2% of participants, stands independently in 2.4% of participants, and walks with assistance in 19.0% of participants; none of the participants were reported to have ever achieved independent walking. This is based on caregiver's report of highest motor milestone that the child ever achieved and may not be the baseline motor milestone. Based on eligibility criteria, all participants had to be able to demonstrate sitting independently, and this was confirmed at baseline by demonstration of sitting independently for at least 10 seconds.

The baseline mean HFMSE total score was 17.97 (range 1.0 to 41.0) in the OAV101B arm, and 18.17 (range 2.0 to 42.5) in the Sham arm, and the mean RULM total score was 16.52 (range 4.0 to 31.7) in the OAV101B arm, and 17.42 (range 4.3 to 31.0) in the Sham arm.

The patients' clinical diagnosis (SMA type) was *not* collected in study B12301.

Confirmatory genetic testing

Confirmatory genetic testing results confirms the absence of functioning *SMN1* gene in all participants. Most participants (119/126, 94.4%) had 3 copies of the *SMN2* gene.

Table 14: Confirmatory genetic testing by treatment at period 1 (FAS)

Characteristic Categories	OAV101B N=75 n (%)	Sham N=51 n (%)	Overall N=126 n (%)
SMN1 copy number			
0 (biallelic deletion)	74 (98.7)	48 (94.1)	122 (96.8)
1	1 (1.3)	3 (5.9)	4 (3.2)
SMN1 copy number=1: variants detected			
Pathogenic	0	1 (2.0)	1 (0.8)
Likely Pathogenic	0	1 (2.0)	1 (0.8)
Variant of uncertain significance	1 (1.3)	1 (2.0)	2 (1.6)
SMN2 copy number			
0	0	0	0
1	0	0	0
2	1 (1.3)	4 (7.8)	5 (4.0)
3	73 (97.3)	46 (90.2)	119 (94.4)
≥4	1 (1.3)	1 (2.0)	2 (1.6)

Percentages are calculated based on the number of participants in the Full analysis set (N).
Confirmatory genetic testing is based on the sample collected during the study.
Pathogenic SMN1 variants were only assessed in participants with one SMN1 copy number.
Source: Study B12301-Table 14.1-2.4.2

Outcomes and estimation

Results for the primary and secondary endpoints are summarized in Table 15 below.

Table 15: Statistical analysis results for primary and secondary endpoints for study B12301 (FAS)

Endpoint	Statistic	OAV101B N=75	Sham N=51	Treatment effect	2-sided p-value	Stat. sig. under testing strategy
Primary endpoint						
HFMSE change from baseline in overall population	LSM (95% CI)	2.39 (1.52 - 3.26)	0.51 (-0.55 - 1.56)	Difference. 1.88 (0.51 - 3.25)	0.0074	Yes
Secondary endpoints						
% HFMSE ≥3-point improvement in overall population	% (95% CI)	39.2 (28.07, 50.31)	26.0 (13.84, 38.16)	OR: 2.03 (0.90, 4.57)	0.0879	No
RULM change from baseline in overall population	LSM (95% CI)	2.44 (1.69 - 3.19)	0.92 (0.00 - 1.83)	Difference: 1.52 (0.34 - 2.71)	0.0122	No
HFMSE change from baseline in 2 to <5 years age group	LSM (95% CI)	3.00 (1.86 - 4.13)	1.56 (0.19 - 2.92)	Difference: 1.44 (-0.33 - 3.22)	0.1097	No
% HFMSE ≥3-point improvement in 2 to <5 years age group	% (95% CI)	48.8 (33.48, 64.08)	37.9 (20.27, 55.59)	OR: 1.27 (0.46, 3.56)	0.6448	No
RULM change from baseline in 2 to <5 years age group	LSM (95% CI)	3.27 (2.20 - 4.33)	1.82 (0.53 - 3.10)	Difference: 1.45 (-0.22 - 3.12)	0.0873	No

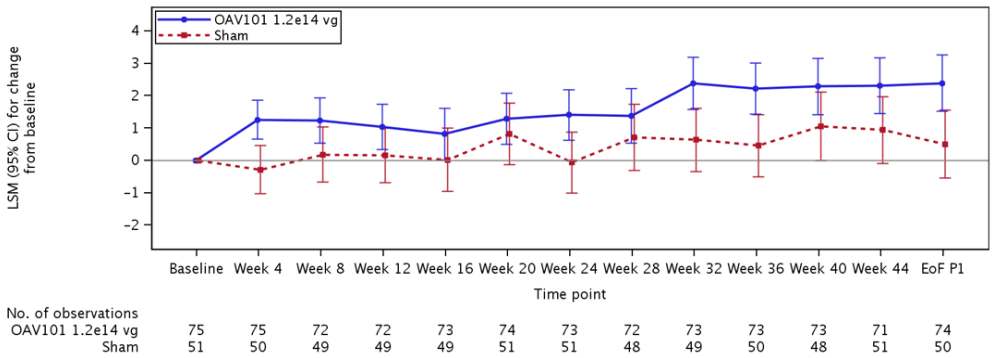
LSM = least square mean, OR = odds ratio

Source: Study B12301 52w-Table 14.2-1.1, Table 14.2-2.1, Table 14.2-3.1, Table 14.2-4.1, Table 14.2-5.1, Table 14.2-6.1

Change from baseline in HFMSE

The primary endpoint was met: the LSM change in HFMSE total score from baseline at the end of Period 1 in the overall population was significantly higher in the OAV101B arm (2.39) than in the Sham arm (0.51), with a LSM difference of 1.88 (95% CI: 0.51 to 3.25, p=0.0074). A difference in change from baseline in LSM HFMSE total score between OAV101B and Sham was observed from Week 4 (first post-dose assessment time point) and remained numerically higher in the OAV101B arm until the end of Period 1 (*Figure 4* below).

Figure 4: Line plot for LSM with 95% CI for change from baseline to the end of Period 1 in HFMSE total score in Study B12301 (overall population) (FAS)

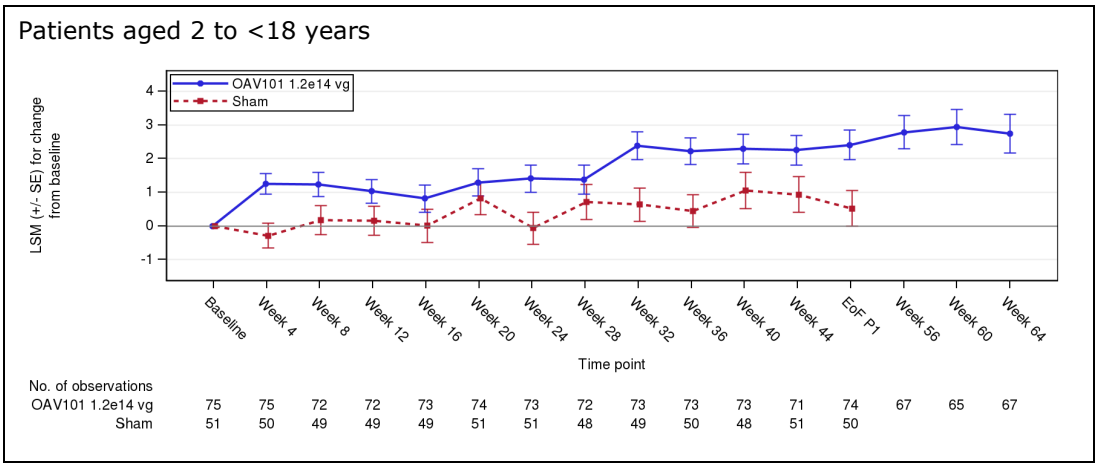


Source: Study B12301 52w-Figure 14.2-1.1

Source: SCE Figure 2-4

Eligible patients who completed the 52-week Period 1 entered Period 2 for an additional 3 months of follow-up. No other SMN-targeted therapies were permitted during the study. A plot of mean HFMSE change from baseline and up to Week 64 for patients who received OAV101B in Period 1 is provided in Figure 5 below.

Figure 5: LSM change from baseline in HFMSE up to week 64 (Period 1 + Period 2) in study B12301 (FAS)



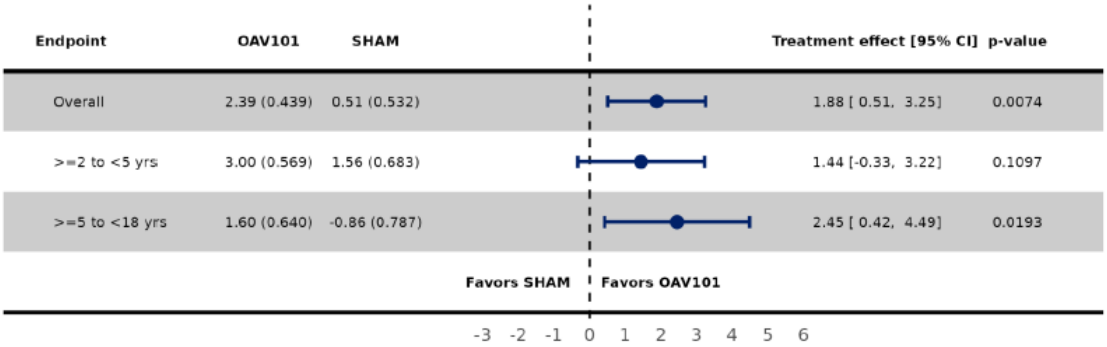
LSM: Least Square Means; SE: Standard Error.
EoF P1: End of Follow-up Period 1 defined as the average of the Week 48 and Week 52 assessments.
Source: [Study B12301 wk64 Suppl-Figure 1-1.post-hoc]

Change from baseline in HFMSE total score by age group

In the 2 to <5 years age group (secondary endpoint), the LSM change from baseline was 3.00 vs 1.56 for OAV101B vs Sham, with a LSM difference of 1.44 (95% CI: -0.33 to 3.22; p-value 0.1097) (Study B12301 52w-Table 14.2-2.1).

In the 5 to <18 years age group (exploratory endpoint), the LSM change from baseline was 1.60 vs −0.86, with a LSM difference of 2.45 (95% CI: 0.42 to 4.49; p-value 0.0193) (Study B12301 52w-Table 14.2-7.1).

Figure 6: Mixed model with repeated measurement analysis of change from baseline in HFMSE to end of Period 1 by age group in study B12301 (FAS)



CI, confidence interval; HFMSE, Hammersmith Functional Motor Scale – Expanded; LSM, least squares mean; SEM, standard error of the mean.
Note: Group estimates are displayed as LSM (SEM) for change from baseline in HFMSE total score at end of Follow-Up Period 1. Treatment effect is displayed as LSM (95% CI).
Source: SCE Figure 2-7

HFMSE ≥3-point improvement overall and by age group

In the overall population (secondary endpoint), the proportion of participants who achieved a ≥3 point increase in HFMSE total score at the end of Period 1 was numerically higher in the OAV101B arm (39.2%) than in the Sham arm (26.0%), with an odds ratio (OR) of 2.03 (95% CI: 0.90 to 4.57; p-value: 0.0879) (table below).

In both the 2 to <5 years age group (secondary endpoint) and the 5 to <18 years age group (post-hoc analysis), the proportion of participants who achieved a ≥3 point increase in HFMSE total score at the end of Period 1 was numerically higher in the OAV101B arm than in the Sham arm.

Table 16: Proportion of participants achieving at least a 3-point improvement from baseline in HFMSE total score at the end of Period 1 for the overall population and by age group in study B12301 (FAS)

Age group Time point	OAV101B $1.2x10^{14}$ vg N=75 n/m (%)	95% CI	Sham N=51 n/m (%)	95% CI	OAV101B $1.2x10^{14}$ vg vs Sham OR	95% CI	p-value*
Overall population End of Follow-up Period 1	29/74 (39.2)	(28.07, 50.31)	13/50 (26.0)	(13.84, 38.16)	2.03	(0.90, 4.57)	0.0879
Participants aged 2 to <5 years End of Follow-up Period 1	20/41 (48.8)	(33.48, 64.08)	11/29 (37.9)	(20.27, 55.59)	1.27	(0.46, 3.56)	0.6448
Participants aged 5 to <18 years End of Follow-up Period 1	9/33 (27.3)	(12.08, 42.47)	2/21 (9.5)	(0.00, 22.08)	3.85	(0.79, 18.66)	0.0940

n=Number of patients achieving at least a 3-point improvement from baseline in HFMSE total score.

m=The total number of patients at each time point.

In the Overall population, the Odds ratio along with 95% confidence intervals for the proportion of patients achieving at least a 3-point improvement from baseline in HFMSE total score is computed using Logistic Regression Model with firth correction including treatment, the strata and baseline HFMSE total score as covariate.

In the 2 to <5 years age group, the Odds ratio along with 95% confidence intervals for the proportion of patients achieving at least a 3-point improvement from baseline in HFMSE total score is computed using Generalized Linear Mixed Effects Model including treatment, visit, treatment by visit interaction, the strata and baseline HFMSE total score as covariate. The model includes "logistic" as the link function. An compound symmetry matrix is used.

In the 5 to <18 years age group, the Odds ratio along with 95% confidence intervals for the proportion of patients achieving at least a x-point improvement from baseline in HFMSE total score is computed using Logistic Regression Model with firth correction including treatment, the strata and baseline HFMSE total score as covariate.

*two-sided p-values.

OR: Odds Ratio.

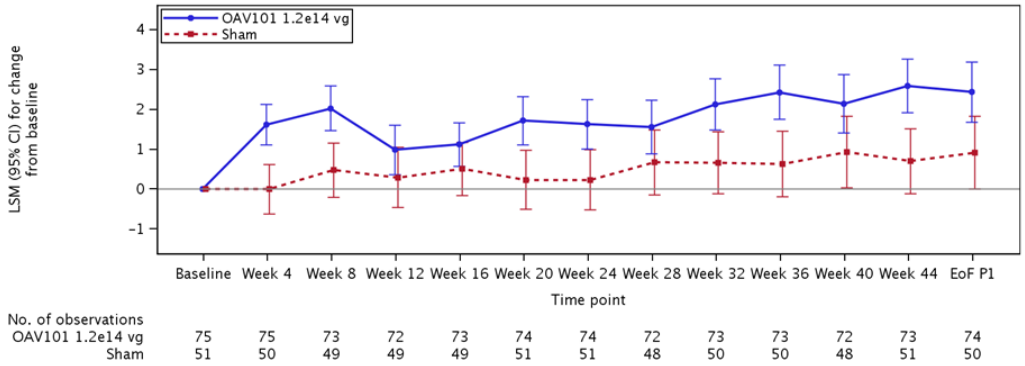
The end of follow-up period 1 assessment is defined as the average of the week 48 and week 52 assessments, assuming both assessments have total scores available. If just one (and not both) of the assessments collected at these two visits has a total score available, then the end of follow-up period 1 assessment is defined as the single assessment total score collected at week 48 or week 52.

Source: SCE-Table 2-6

Change from baseline in RULM total score in the overall population

In the overall population, the LSM change from baseline to end of Period 1 in RULM total score was 2.44 vs 0.92, with a LSM difference of 1.52 (p-value=0.0122) for OAV101B vs Sham. Based on the prespecified multiple testing strategy, the difference was not statistically significant. The LSM change from baseline in RULM total scores was numerically higher in the OAV101B arm than in the Sham arm from Week 4 and until the end of Period 1 (Study B12301 52w-Table 14.2-5.1).

Figure 7: Line plot for LSM with 95% CI for change from baseline in RULM total score in study B12301 (overall population) (FAS)



Source: Study B12301 52w-Figure 14.2-1.4

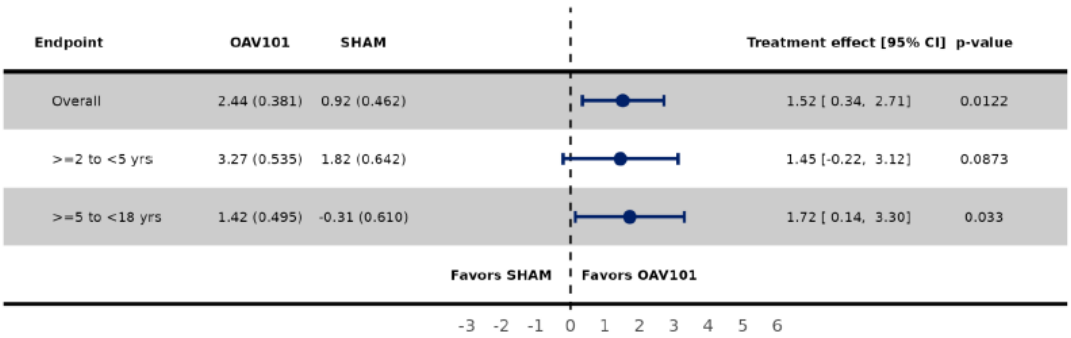
Change from baseline in RULM total score by age group

Analysis of change in RULM total score shows numerically higher improvement in the OAV101B arm compared to the Sham arm in both the 2 to <5 years age group and the 5 to <18 years age group.

In the 2 to <5 years age group (secondary endpoint), at the end of Period 1, the LSM change from baseline in RULM total score was 3.27 vs 1.82 in the OAV101B vs Sham arms, with a LSM difference of 1.45 (95% CI: -0.22 to 3.12, p-value 0.0873) (Study B12301 52w-Table 14.2-6.1).

In the 5 to <18 years age group (exploratory endpoint), the LSM change from baseline was 1.42 vs −0.31 in the OAV101B vs Sham arms, respectively, with a LSM difference of 1.72 (95% CI: 0.14 to 3.30, p-value 0.0330) (Study B12301 52w-Table 14.2-7.2).

Figure 8: Forest plot of RULM change from baseline in study B12301 by age groups (FAS)



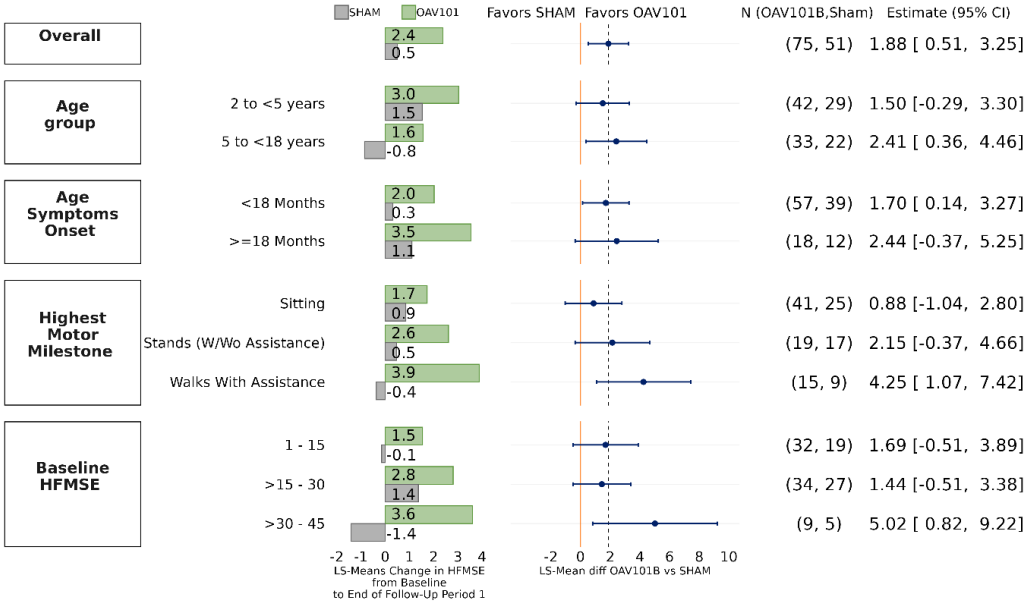
CI, confidence interval; LSM, least squares mean; RULM, Revised Upper Limb Module; SEM, standard error of the mean.
Note: Group estimates are displayed as LSM (SEM) for change from baseline in RULM total score at end of Follow-Up Period 1. Treatment effect is displayed as LSM (95% CI).
Source: SCE Appendix 1-Figure 2-6exp

Pre-defined and post-hoc subgroup analyses

Post-hoc subgroup analyses of the primary endpoint (HFMSE change from baseline at the end of Period 1) were performed by age group (2 to <5 and 5 to <18 years), motor function level (HFMSE total score, highest motor milestone ever achieved prior to baseline), and age at symptom onset (<18 months of age, ≥18 months of age) which can differentiate between Type 2 and Type 3 SMA. Result are displayed in Figure 9 below, derived from treatment by subgroup interaction models.

No subgroup analyses were conducted for RULM.

Figure 9: Subgroup analysis of the primary endpoint in study B12301 (FAS)



The efficacy endpoint variable is analyzed using a linear mixed effects repeated measures model (MMRM) with the observed change from Baseline in HFMSE total score at all post-baseline visits as the dependent variable.
The fixed effects include treatment, visit, treatment by subgroup by visit interaction, strata and baseline HFMSE total score as covariate. An unstructured covariance matrix is used.
The vertical dotted line represents the treatment effect in the overall population.
Source: [SCE Appendix 1-Figure 2-9exp]

Ancillary analyses

HFMSE ≥1.5-point improvement overall and by age group

A post-hoc analysis was conducted using a threshold of 1.5 point for HFMSE improvement for the overall population, and the 2 to <5 years and 5 to <18 years age groups. Coratti et al. (2024) established an MCID of 1.46 for improvement in HFMSE in patients with type 2 SMA by using an anchor-based methodology in accordance with FDA guidance (FDA 2023). Results are presented in Table 17 below.

Table 17: Proportion of participants achieving at least a 1.5-point improvement from baseline in HFMSE total score at the end of period 1 for the overall population and by age group in study B12301 (FAS)

	OAV101B $1.2x10^{14}$ vg N=75		**Sham N=51**		**OAV101B $1.2x10^{14}$ vg vs Sham**		
Age group Time point	**n/m (%)**	**95% CI**	**n/m (%)**	**95% CI**	**OR**	**95% CI**	**p-value***
Overall population End of Follow-up Period 1	47/74 (63.5)	(52.55, 74.48)	21/50 (42.0)	(28.32, 55.68)	2.83	(1.28, 6.24)	0.0102
Participants aged 2 to <5 years End of Follow-up Period 1	30/41 (73.2)	(59.61, 86.73)	17/29 (58.6)	(40.70, 76.55)	1.83	(0.66, 5.09)	0.2466
Participants aged 5 to <18 years End of Follow-up Period 1	17/33 (51.5)	(34.46, 68.57)	4/21 (19.0)	(2.25, 35.84)	5.69	(1.43, 22.57)	0.0134

n=Number of patients achieving at least a 1.5-point improvement from baseline in HFMSE total score.

m=The total number of patients at each time point.

In the Overall population, the Odds ratio along with 95% confidence intervals for the proportion of patients achieving at least a 1.5-point improvement from baseline in HFMSE total score is computed using Logistic Regression Model with firth correction including treatment, the strata and baseline HFMSE total score as covariate.

In the 2 to <5 years age group, the Odds ratio along with 95% confidence intervals for the proportion of patients achieving at least a 1.5-point improvement from baseline in HFMSE total score is computed using Logistic Regression Model with firth correction including treatment, the strata and baseline HFMSE total score as covariate.

In the 5 to <18 years age group, the Odds ratio along with 95% confidence intervals for the proportion of patients achieving at least a 1.5-point improvement from baseline in HFMSE total score is computed using Logistic Regression Model with firth correction including treatment, the strata and baseline HFMSE total score as covariate.

*two-sided p-values.

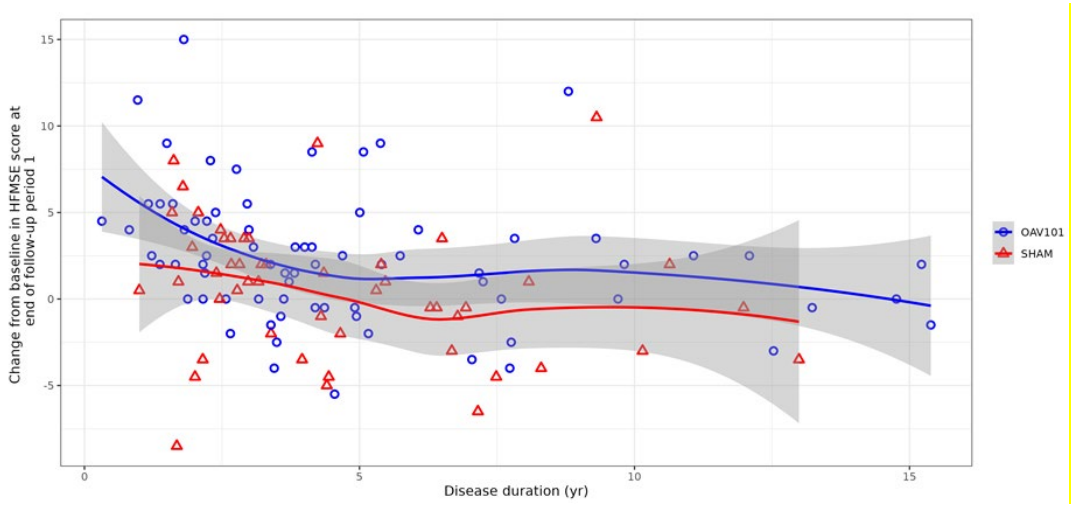
OR: Odds Ratio.

The end of follow-up period 1 assessment is defined as the average of the week 48 and week 52 assessments, assuming both assessments have total scores available. If just one (and not both) of the assessments collected at these two visits has a total score available, then the end of follow-up period 1 assessment is defined as the single assessment total score collected at week 48 or week 52.

Source: SCE-Table 2-7

The effect of disease duration (calculated as age at treatment initiation - age at symptom onset) on treatment response was evaluated as shown in Figure 10.

Figure 10: Scatter plot of disease duration vs change from baseline in HFMSE total score at end of follow-up period 1 using LOESS in study B12301 (B12301 full analysis set)



LOESS = locally estimated scatter plot smoothing
The bold line represents the LOESS-smoothed trend. The shaded area indicates the 95% confidence interval around the estimated trend.
Disease duration = Age at dosing (yr) - age at symptom onset (yr).
Source: [COAV101B1-EU HAQ2-Figure 6-2.2.haq-eu2]

5.3.4. Clinical studies in special populations

All participants included in the OAV101B clinical trials were paediatric (<18 years of age). OAV101B has not been studied in patients with hepatic or renal impairment.

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

Data from participants who received OAV101B in the sham-controlled Period 1 of Study B12301 (n=75) and participants who received the therapeutic dose of OAV101B $1.2x10^{14}$ vg and were ≥2 years of age in Study CL-102 (n=12) were pooled for evaluation of efficacy of OAV101B through 52 weeks. No hypothesis testing was performed.

In the pooled population (n=87), the mean change from baseline at Week 52 in HFMSE total score was 2.97 (range -6.0 to 23); 39/87 participants (45.3%) had an increase in HFMSE total score of ≥ 3 points, and 57/87 participants (66.3%) had an increase of ≥ 1.5 points (SCE Appendix 1-Table 2-1, Table 2-2, Table 2-3.2).

5.3.6. Supportive studies

Supportive data for efficacy of OAV101B are provided by studies CL-102 and B12302.

For study CL-102, it is referred to section 6.3.1 of this report.

Study B12302 Phase IIIb, open-label, single-arm, multi-center study to evaluate the safety, tolerability and efficacy of OAV101 administered intrathecally (1.2x1014 vector genomes) to

participants 2 to <18 years of age with spinal muscular atrophy (SMA) who have discontinued treatment with nusinersen (Spinraza®) or risdiplam (Evrysdi®)

This was a Phase IIIb, 12 months, open-label, single arm, multi-center study to evaluate the safety, tolerability and efficacy of OAV101B in participants with SMA aged 2 to <18 years after the discontinuation of treatment with nusinersen or risdiplam.

Study population

Eligible patients were 2 to <18 years of age, had any *SMN2* copy number, and onset of clinical signs and symptoms of SMA at <18 months of age or diagnosis via newborn screening. They had to be able to sit independently and had never walked independently.

Description of trial intervention

OAV101B was administered by lumbar IT injection as a single dose of $1.2x10^{14}$ vg. Participants received immunomodulatory therapy with prednisolone, using the same dosing regime as for Study B12301.

Objectives, endpoints, and estimands

The primary and secondary objectives of Study B12302 are shown in Table 18 below.

Table 18: Objectives and related endpoints in study B12302

Objective(s)	Endpoint(s)
Primary objective(s)	**Endpoint(s) for primary objective(s)**
To characterize the safety and tolerability of OAV101 IT over a 52-week period in patients with SMA aged 2 to < 18 years who have discontinued treatment with nusinersen (Spinraza®) or risdiplam (Evrysdi®).	Number and percentage of participants reporting AEs, related AEs, SAEs, and AESIs
Secondary objective(s)	**Endpoint(s) for secondary objective(s)**
To assess the efficacy of OAV101 IT on motor function, and caregiver impact over a 52-week period in patients with SMA aged 2 to < 18 years who have discontinued treatment with nusinersen (Spinraza®) or risdiplam (Evrysdi®)	• Change from baseline to Week 52 visit in the HFMSE total score • Change from baseline to Week 52 visit in the RULM total Score • Change from baseline to Week 52 visit in Assessment of Caregiver Experience in ACEND instrument score

Statistical methods

The full analysis set (FAS) included all enrolled participants who received OAV101B, and was used for both the efficacy and the safety analyses. No hypothesis testing was performed.

Results

Study population

Study B12302 enrolled 27 treatment experienced participants with SMA who were able to sit, but had never been able to walk independently prior to enrollment. The mean age was 7.4 years (range 2.4 to 17.7 years), 55.6% were male and 44.4% were female. Whites accounted for 48.1% of participants, Asian for 22.2%, and 29.6% of participants were other races or did not report their race (Study B12302-Section 10.4).

The mean age of symptoms onset was 0.784 years (range 0.08-1.42 years), and the mean age at diagnosis was 1.05 years (range 0.11 to 1.93 years). All participants were symptomatic at enrollment, and the most frequent symptoms were limb weakness (96.3%) and hypotonia (70.4%). The majority of participants (85.2%) had 3 copies of *SMN2*; 14.8% had 2 copies of *SMN2*.

The highest motor function ever achieved prior to study entry was sitting without support for 63.0% of participants, standing with assistance for 18.5% of participants, and walking with assistance for 18.5% of participants. None of the participants had the ability to walk independently prior to enrollment. Similar to Study B12301, this is based on caregiver's report of highest motor milestone that the child ever achieved, and may not be the baseline motor milestone. Based on eligibility criteria, all participants were able to sit independently.

All participants had received prior SMN-targeted treatment: 77.8% had received nusinersen (mean duration of 4.3 years; range 1.86 to 6.18 years), 14.8% had received risdiplam (mean duration of 3.0 years; range 0.39 to 6.28 years), and 7.4% had received both but not concurrently (Study B12302-Section 10.5).

N=6 out of the 27 enrolled patients discontinued the most recent previous SMN-targeted therapy due to lack of efficacy as reported by the caregivers (nusinersen in 5 patients, risdiplam in 1 patient). For the remaining 21 patients the reason was given as "other", as these patients discontinued their treatment to be eligible for enrolment into study B12302.

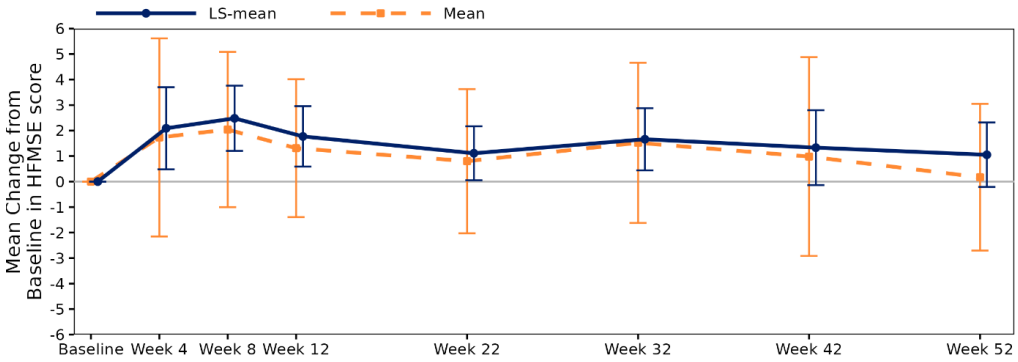
At baseline, the mean HFMSE total score was 24.31 and ranged from 2.0 to 46.5, and the mean RULM total score was 24.60 and ranged from 7.5 to 36.0 (Study B12302-Section 11.1).

Efficacy results

The results for the secondary and exploratory efficacy endpoints HFMSE and RULM total scores up to Week 52 were as follows (Study B12302-Section 11.1):

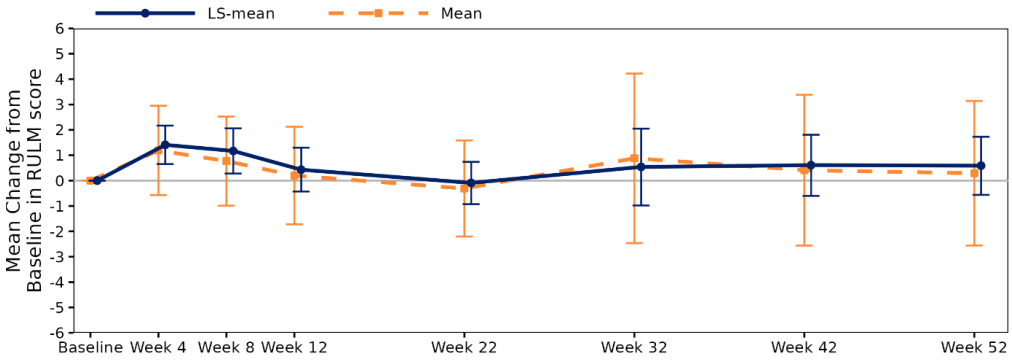
- The mean change from baseline to Week 52 in HFMSE total score was 0.17 (range -5.5 to 7.5), and the LSM change from baseline to Week 52 was 1.05 (95% CI: -0.21 to 2.32).
- The mean change from baseline to Week 52 in RULM total score was 0.29 (range -6.0 to 6.0), and the LSM change from baseline to Week 52 was 0.59 (95% CI: -0.56 to 1.73).

Figure 11: Line plots for change from baseline to Week 52 in HFMSE total score for overall group by visit in study B12302 (full analysis set)



Least squares mean (solid line) ± 95 % CI (error bars)
Means (dotted line) ±SD (error bars)
Source: Study B12302-Figure 11-1

Figure 12: Line plots for change from baseline to Week 52 in RULM total score for overall group by visit (full analysis set)



Least squares mean (solid line) ± 95 % CI (error bars)
Means (dotted line) ±SD (error bars)
Source: Study B12302-Figure 11-2

Subgroup analyses of Study B12302

Post hoc subgroup analyses of HFMSE change from baseline were conducted to examine the treatment effect of OAV101B in participants with symptoms onset before or at 6 months of age, which differentiates between the SMA Type 1 and Type 2 classification (Arnold et al 2015). The results are shown in Table 19 below.

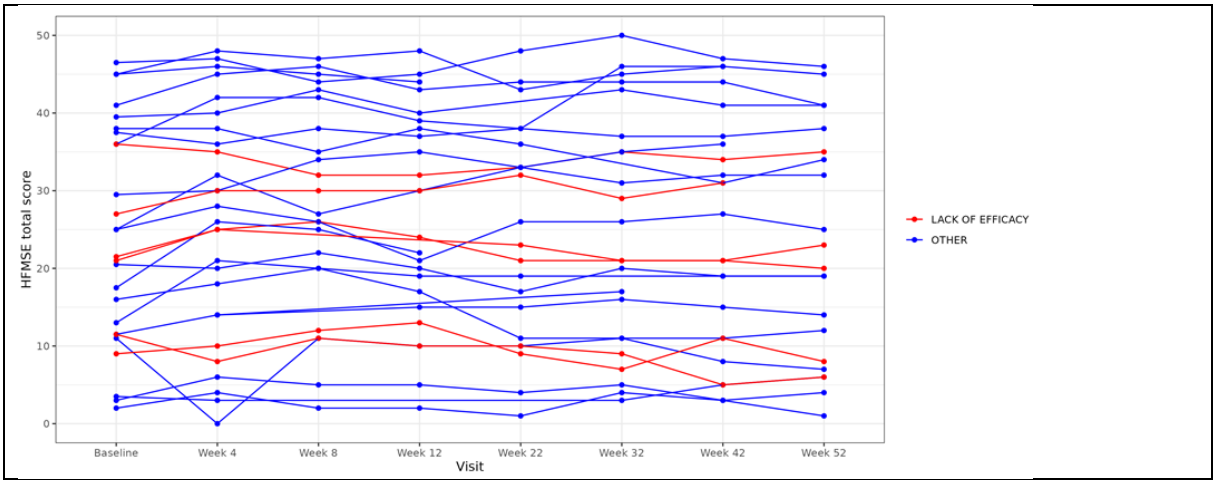
Table 19: HFMSE change from baseline in Study B12302 by age of symptoms onset (B12302 full analysis set)

	Age at symptoms onset					
	≤6 months		**7 to ≤18 months**		**Overall**	
	N	**Means (SD)**	**N**	**Means (SD)**	**N**	**Means (SD)**
Week 4	7	2.36 (3.59)	19	1.50 (4.06)	26	1.73 (3.89)
Week 8	6	3.67 (2.70)	17	1.47 (3.02)	23	2.04 (3.04)
Week 12	7	2.79 (2.23)	17	0.71 (2.70)	24	1.31 (2.70)
Week 22	6	1.50 (2.17)	16	0.53 (3.06)	22	0.80 (2.83)
Week 32	5	1.00 (2.65)	17	1.68 (3.33)	22	1.52 (3.14)
Week 42	6	0.33 (3.72)	18	1.19 (4.03)	24	0.98 (3.90)
Week 52	6	0.33 (3.3)	15	0.10 (2.82)	21	0.17 (2.88)

Source: SCE Appendix 1-Table 4-2exp

Post hoc subgroup analyses of HFMSE total score in patients who discontinued prior SMN-targeted therapy due to lack of efficacy as reported by caregivers are displayed in the figure below (n=6, red lines).

Figure 13: Spaghetti plot of HFMSE to Week 52 by reason for discontinuing prior SMN-targeted therapy in study B12302 (B12302 full analysis set)



5.3.7. Long-term follow-up studies

Study design

Participants who received OAV101B in a clinical study were, after completing the study, could enroll into a long-term follow-up (LTFU) study, where they were followed for efficacy and safety evaluations as follows:

- Participants from studies B12301 and B12302 could enroll in study COAV101A12308 (Study A12308).
- Participants from Study CL-102 could enroll into the long-term study AVXS-101-LT-002 (Study LT-002).

Study A12308 is comprised of a Baseline Period and 2 Follow-up (FU) Periods. In the first 2 years (FU Period 1), visits occur every 6 months and thereafter (FU Period 2) visits are conducted annually. The total duration of FU in Study A12308 is 5 years after enrollment in the study (the duration of FU was reduced from 15 years to 5 years after enrollment into the study with amended protocol version 04, dated 01-Aug-2024).

Study LT-002 comprised 2 phases, an initial 5-year FU phase, with visits every 6 months for Years 0 to 2 and annually for Years 3 to 5, followed by an observational phase during which participants are contacted annually via telephone. The total FU duration is 15 years post OAV101B dosing in the parent study.

Treatment with SMN-targeted therapy (nusinersen or risdiplam) was allowed during all LTFU studies.

All LTFU studies are currently ongoing.

Objectives and endpoints

The primary and secondary objectives in the LTFU studies were:

- Study A12308: primary objective was long-term safety, long-term efficacy was secondary objective.
- Study LT-002: primary objective was long-term follow-up safety and efficacy.

Both LTFU studies included HFMSE and RULM as efficacy assessments. In Study LT-002, RULM was added with amendment 4 dated 27-Aug-2021, thus data were too limited at the data cut-off (24-Jun-2024) for analysis.

Statistical methods

The efficacy endpoints evaluated in Study A12308 and LT-002 were HFMSE change from baseline, and (in A12308 only) RULM change from baseline. The first assessment in the LTFU study served as baseline, and could be later than the actual baseline visit. Change from baseline in HFMSE and RULM total scores were summarized descriptively. No hypothesis testing was performed.

Results

Study A12308

At the time of the data cut-off for submission for MA (05-Nov-2024), 59 participants who received OAV101B ($1.2x10^{14}$ vg) enrolled in Study A12308 (46 from Study B12301 and 13 from Study B12302). However, the majority of these participants had not yet reached Month 6, the first post-baseline assessment timepoint for HFMSE and RULM. Furthermore, as Study B12301 was ongoing at the time of data cut-off, randomized allocation to study treatment (i.e. OAV101B or Sham) was blinded for Study B12301, therefore it was not possible to differentiate between participants who had received OAV101B in Period 1 (corresponding to 52 weeks of FU) vs in Period 2 (corresponding to 12 weeks of FU post-dosing), or to calculate the time of FU since OAV101B dosing (SCE-Section 2.6).

Ten participants who enrolled from Study B12301 had reached Month 6 and had both baseline and post-baseline HFMSE and RULM total scores. The mean change from baseline to Month 6 in HFMSE total score was 1.0, and ranged from −5 to 5; for RULM total score, the mean change from baseline was 0.5, and ranged from −7 to 7.

Only 3 participants who enrolled from Study B12302 had both a baseline and a post-baseline HFMSE assessment in Study A12308. Their post-baseline scores ranged from a 12-point decrease to a 2-point increase compared to baseline.

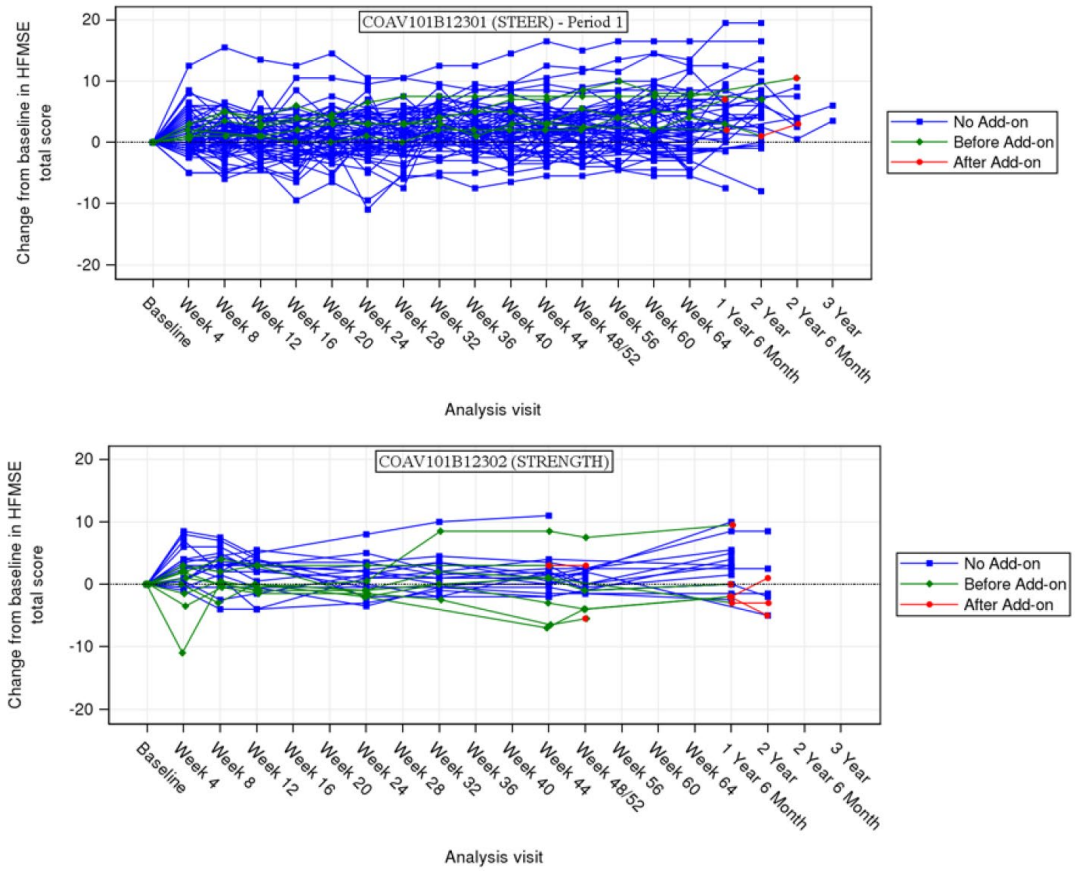
SMN-targeted therapies (nusinersen and risdiplam) are allowed in Study A12308.

Updated long-term follow-up data were subsequently provided from a more recent data cut-off (24-Jun-2025). At this time, the study had enrolled a total of 91 patients who had received OAV101B (71 from B12301, and 20 from B12302), and 61 of these patients (45 from B12301 and 16 from B12302) had at least one post-baseline HFMSE assessment (6 months or beyond) in Study A12308. The median follow-up in this analysis was 1.91 years (range 0.6 to 2.9) from the time of dosing in the parent study (for patients from Study B12301, this was either OAV101B or Sham administered in Period 1).

Of the 91 OAV101B-treated patients enrolled into study A12308, 15 had received SMN-targeted treatment during study A12308: 5 received nusinersen, and 10 received risdiplam.

Patient-level profiles exploring the timing of introduction of other SMN-targeted therapies on the HFMSE trajectories from parent study baseline (B12301 or B12302) to the most recent assessment in study A12308 are presented in Figure 14 below.

Figure 14: Individual longitudinal trajectories for change from baseline in total score of Hammersmith Functional Motor Scale – Expanded (HFMSE) by add-on usage – time plots (OAV101 pooled analysis set)



Study LT-002

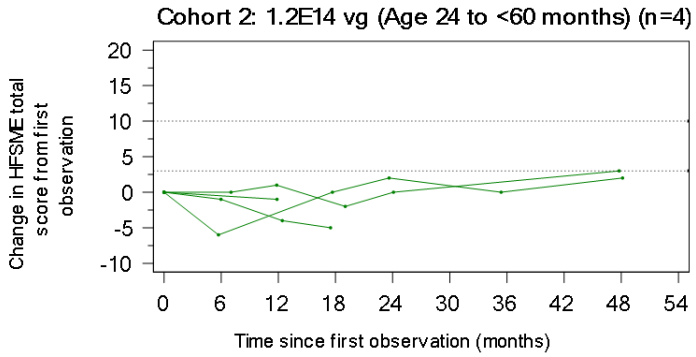
At the time of data cut-off for submission for MA (24-Jun-2024), 18 participants received OAV101B in Study CL-102. Among these 18 participants, 12 participants (8 in the 6 to <24 months age group, and 4 in the 24 to <60 months age group) had received the proposed therapeutic dose of $1.2x10^{14}$ vg. (SCE-Section 2.5).

24 to <60 months age group

In the 24 to <60 months of age group (n=4), the median FU time since OAV101B dosing in the parent study was 5.86 years (range 4.4 to 5.9 years). Three of the 4 participants were ongoing, and had reached their 4-year assessment in Study LT-002 (one of these participants had HFMSE data at the Year 3 and Year 4 visits only; therefore the Year 3 visit was taken as baseline). The 4th participant discontinued at Month 18.

All participants received other SMN-targeted therapies (nusinersen or risdiplam) during Study LT-002.

Figure 15: Individual longitudinal trajectories for change from baseline in HFMSE total score for participants aged 24 to <60 months at the time of dosing with 1.2x1014 vg OAV101B - study LT-002 (all enrolled participants)



For HFMSE, baseline is defined as the first observed score in the study per participant and may not necessarily be the same as the time of study start.
A total score is not derived if any item score is missing or cannot test at baseline or post-baseline visit.
n is the number of participants with the HMSE total score calculated both at baseline and post-baseline.
Horizontal reference lines correspond to change of the total score equal to 3 or 10.
Source: Study LT-002-Figure 14.2-1.2a

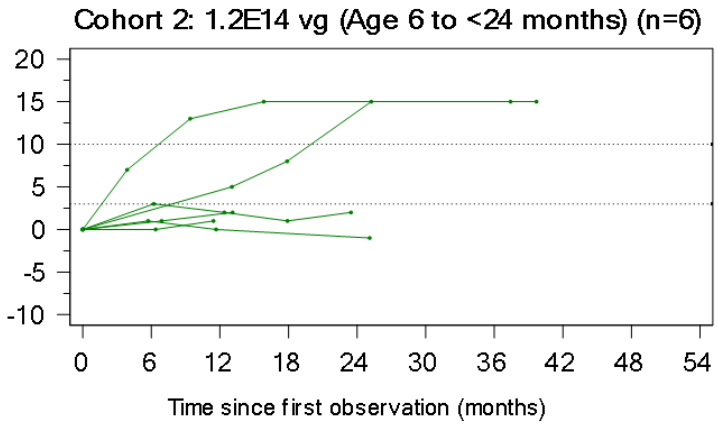
6 to <24 months age group

In the 6 to <24 months age group (n=8), the median FU time since dosing in the parent study was 5.27 years (range 2.9 to 6.2) at the time of data cut-off, and 4/8 participants were ongoing. Three participants discontinued due to withdrawal of consent, and one was lost to FU. 5/8 participants received SMA therapy (risdiplam) during Study LT-002.

6/8 participants had a baseline and at least one post-baseline HFMSE total score reported during LT-002. The mean (SD) HFMSE total score at baseline was 27.8 (16.35) (range 8 to 51), and the mean (SD) change in HFMSE total scores from baseline to the most recent assessment was 5.7 (7.31).

Two participants had a marked increase in HFMSE total score from baseline to the most recent assessment: from 24 at baseline to 39 at Years 2 and 3 (+15 from baseline) in one participant, and from 51 at baseline to 66 at Years 2, 3 and 4 (+15 from baseline) in the other participant (Figure 16 below).

Figure 16: Individual longitudinal trajectories for change from baseline in HFMSE total score for participants aged 6 to <24 months at the time of dosing with 1.2x1014 vg OAV101B - study LT-002 (all enrolled participants)

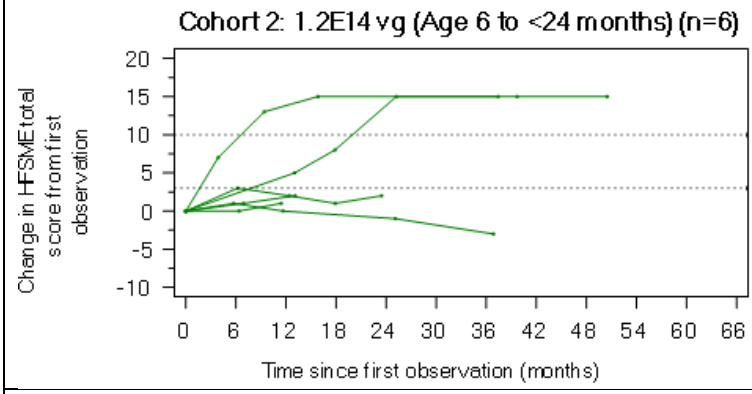


For HFMSE, baseline is defined as the first observed score in the study per participant and may not necessarily be the same as the time of study start.
In case a participant is younger than 24 months of age at the time of the baseline visit, the HFMSE total score at the earliest visit after 24 months of age is reached is considered as baseline.

A total score is not derived if any item score is missing or cannot test at baseline or post-baseline visit.
n is the number of participants with the HMSE total score calculated both at baseline and post-baseline.
Horizontal reference lines correspond to change of the total score equal to 3 or 10.
Source: Study LT-002-Figure 14.2-1.2a

Updated follow-up data were subsequently provided from a more recent cut-off date (30-Jun-2025). Data are displayed in the figures below. Other SMA-targeted therapy was reported for 5 of the 8 patients enrolled from the 6 to <24 month cohort, and all 4 patients who enrolled from the 24 to <60 month cohort.

Figure 17: Individual longitudinal trajectories for change from baseline in HFMSE in study LT-002 – patients who received OAV101B at the therapeutic dose in study CL-102



Source: [Study LT-002 Figure 14.2-1.2a]

5.3.8. Patient / caregiver engagement

To better understand the impact of SMA on individuals and caregivers and the unmet medical need, 1:1 interviews were conducted with caregivers of individuals with SMA. The results are presented in the SMA Caregiver Experience Report. These interviews (n=8) pointed to the following burden with respect to life-long treatment with nusinersen or risdiplam:

- Financial and insurance coverage stress with obtaining pre-authorization and continuation of medical refills
- Maintenance logistics burden in scheduling appointments and commute for IT administration of nusinersen, and ensuring timely delivery of medication to home and refrigeration needs for risdiplam oral solution
- Challenges with administration of IT medication (nusinersen) every 4 months with increasing age and burden due to scoliosis and emotional strain for the patients during these procedure
- Parents / caregiver fear for the future due to uncertainty if their child with SMA will continue to prioritize treatment maintenance when they are adults and/or are living on their own, or if insurance will continue to cover these life-long treatments.

In addition, as an exploratory objective of study B12302, semi-structured qualitative interviews were conducted with caregivers of patients at two different time points: at the study entry (pre-treatment), and within 4 weeks of the patient's end-of study visit (post-treatment). Twenty-two caregivers were interviewed at the pre-treatment stage, and 20 caregivers were interviewed post-treatment.

Variables of interest were responses to open-ended interview questions designed to elicit information about the patient's experience of SMA (i.e., symptoms and daily life impacts) from the caregiver's

perspective, caregiver's experience of caring for a patient with SMA, expectations of realistic treatment outcomes after administration of OAV101B, experiences with previous treatments for SMA, changes in symptom experience and daily life impacts (both patient and caregiver) during the clinical study and their meaningfulness, and treatment perspectives for OAV101B.

Results of this study showed that caregivers' overall satisfaction with the OAV101B treatment and the treatment outcomes varied, as a function of the patient's study outcomes, differences in the caregivers' experiences of caring for someone with SMA, and treatment expectations before the study.

5.3.9. Overall discussion and conclusions on clinical efficacy

5.3.9.1. Discussion

Design and conduct of clinical studies

The originally proposed indication of OAV101B (Itvisma) was:

"… for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene in patients 6 months of age and older."

The proposed posology is a single fixed dose of 1.2×10^{14} vg for the whole proposed target population.

In support of the proposed indication and posology, 5 studies were included in the dossier, of which 3 completed (B12301, CL-102, B12302) and 2 ongoing (LT-002, A12308). Pivotal data for efficacy derive from study B12301, the other studies are regarded as supportive.

Study CL-102 was a Phase I/II, dose-ranging, open label study conducted in treatment-naïve patients with SMA aged 6 to <60 months.

Study B12301 was a Phase III, randomized, sham-controlled, double-blind, multi-center study conducted in treatment-naïve patients with SMA aged 2 to <18 years.

Study B12302 was a Phase IIIb, open label study conducted in patients with SMA aged 2 to <18 years who were previously treated with nusinersen or risdiplam.

After completion of the study, participants from CL-102 could enroll into long-term follow-up (LTFU) study LT-002, while participants from B12301 and B12302 could enroll into LTFU study A12308.

Dose finding supportive study

Study **CL-102** was an open-label, single-dose, dose ranging study conducted in 32 treatment-naive patients aged 6 months to 5 years, with 3 *SMN2* copies, age of symptom onset less than 12 months, and able to sit but not to walk independently. These criteria correspond to SMA patients type 2.

Patients were stratified into two age groups at time of dosing: 6 to <24 months, and 24 to <60 months. Efficacy was assessed separately in each age group using age-appropriate instruments. Primary endpoint (EP) for the 6-24 month cohort was achieving the motor milestone "standing alone" up to 12-months, and for the 24-60 month cohort was "change from baseline in HFMSE at 12-months". Both primary EP were compared to a natural history cohort from the PNCR dataset, matched for eligibility criteria, which is adequate.

Three incremental doses of OAV101B were tested in study CL-102: dose A (6.0×10^{13} vg, n=3), dose B (1.2×10^{14} vg, n=25) and dose C (2.4×10^{14} vg, n=4).

Dose A (low dose) was received by 3 patients aged ≥6 - <24 months, before escalating to dose B (intermediate dose) which was received by 13 patients in the age group ≥ 6 - <24 months and 12 patients in the age group ≥24 - <60 months. Due to dorsal root ganglia (DRG) toxicity findings in a non-human primate (NHP) study (ITFS-101, see NC AR), study CL-102 was suspended by the FDA (29-Oct-2019) when 4 patients aged ≥6 - <24 months received dose C (the highest dose). After review of the NHP data, the partial hold was lifted (30-Jul-2021), no further participants were enrolled in the high dose cohort. The intermediate dose was selected as therapeutic dose.

Pivotal study

Study **B12301** was a 52-week, phase 3, multi-center, randomized (3:2) double-blind, sham-controlled study that enrolled 126 SMA patients (OAV101B =75, Sham=51), aged ≥2 to <18 years, symptom onset (as based on caregivers' reports) between 6 and 33 months, able to sit but never able to walk independently, and with mostly (94%) 3 copies of the *SMN2* gene. The participants' SMA type was not collected in the study.

Study B12301 consisted of two periods: a 52-week Period 1, and a 12-week Period 2 (i.e., up to Week 64). In Period 1, participants were randomized 3:2 to receive OAV101B or sham, and then followed-up for 52 weeks. Thereafter, eligible participants entered Period 2. Patients who received a sham procedure in Period 1 received OAV101B in Period 2, and vice-versa. Pivotal data derive from Period 1.

Main eligibility criteria were: no prior SMA-targeted treatment (e.g., risdiplam and nusinersen); genetically confirmed biallelic SMN1 pathogenic variants; symptoms onset at ≥6 months of age, ability to sit independently but not to walk independently. Patients were able to receive a lumbar puncture and to perform motor function assessments (i.e., no severe scoliosis or contractures).

The employed exclusion criteria were sufficient to not expose study participants to unnecessary safety concerns. In particular, patients with sensory abnormalities in the neurological examination / sensory nerve action potential (SNAP) were excluded.

Patients were also excluded in case of elevated anti-AAV9 antibody titers at screening, with reference to >1:50 or a validated result consistent with being elevated. The three anti-AAV9 assays employed in the study were developed to either meet the requirement of the > 1:50 exclusion titer , or had demonstrated analytical equivalence with the 1:50 titer. Therefore, the utilized different assays were comparable in determining the participants' eligibility based on the 1:50 antibody titer cut-off.

The 1:50 cut-off was the same employed for Zolgensma clinical trials, although the immune response was expected to be lower with IT injection compared to IV. The Applicant clarified that the chosen anti-AAV antibody cut-off was agreed upon with the FDA for the Zolgensma program based on abundance of caution, and not as a consequence of expected efficacy or safety risks. The same 1:50 criterion was then applied directly to the OAV101B program, and arguably based on even higher caution as antibody titers are lower in the CSF compared to blood, and as high pre-existing serum anti-AAV9 antibodies did not impact biodistribution to target tissue or efficacy in the nonclinical studies (NC report, 3.3.1). In fact, a number of AAV IT gene therapies trials did not exclude patients based on pre-existing serum anti-AAV antibodies. Therefore, theoretically, elevated serum anti-AAV9 antibodies do not represent a significant risk for efficacy or safety of OAV101B. However, in practice, this risk has not been excluded clinically, as all the OAV101B clinical studies employed the 1:50 cut-off. Therefore, a potential risk in patients with elevated serum anti-AAV9 antibodies remains, which has been adequately reflected with a warning in the SmPC.

Participants randomized to the OAV101B arm received prophylactic immunomodulatory therapy with prednisolone, intended to mitigate safety risks associated with the immune response against the capsid. The prednisolone regimen employed for OAV101B (1mg/kg/day 1 day prior the injection and

the following 30 days, tapered in 28 days) was the same as the one employed for Zolgensma. Again, the proposed corticosteroid regimen for OAV101B derives directly from the Zolgensma program, although the immune response was expected to be lower with OAV101B, given the lower total vector loads and the reduced systemic exposure. However, in the absence of data, no different dosages or immunomodulatory therapies can be recommended. For clarity, additional text on the tapering scheme was added to section 4.2 of the SmPC.

The sham procedure consisted of a needle prick that broke the skin, but without performing lumbar puncture. In scientific advice, the Applicant indicated that patients in the sham arm would receive a lumbar puncture with CSF collection for baseline studies in order to mitigate risk of unblinding from post-lumbar puncture headache. This would have been preferable for blinding purposes. The sham procedure with a needle prick was chosen instead by the Applicant for risk minimisation purposes, which is acceptable.

After OAV101B injection, participants were placed in Trendelenburg position. As demonstrated in NHP studies, this is important to enhance distribution to cervical and brain regions, hence has been specified in the method of administration section of the SmPC.

Primary endpoint (EP) was change-from-baseline in the Hammersmith Functional Motor Scale – Expanded (HFMSE) motor function score at week 52. A key secondary EP was the RULM (Revised Upper Limb Module) Score. The HFMSE and RULM are two complementary and validated scales in SMA, appropriate to assess clinically relevant effects in the included 2 to <18 years population. These study efficacy assessments are considered sufficient for the intended purpose. While both methods are clinically validated, it is important to mention that RULM mainly measures motor performance in the upper limbs and patients with all forms of SMA have diffuse symmetric proximal muscle weakness that is greater in the lower than upper limbs. Therefore, the choice of HFMSE change as a primary endpoint is justifiable. A change in the HFMSE score ≥3 points, and in the RULM score ≥2 points, are considered clinically meaningful (Mercuri et al., 2018). Accordingly, HFMSE responder analyses were included as secondary EP. Secondary analyses in participants aged 2 to <5 years were included to ensure that, in case of failure of the primary EP in the whole population, treatment effect could be characterized in the younger age group. It must be noted that a large proportion of the alpha in the graphical testing procedure was initially assigned to the treatment effect on the change in HFMSE in those aged 2 to <5 years and that the study was powered for this endpoint in the younger population where a larger effect (5 points) on this endpoint was assumed as compared to the overall population (and which if present would require the effect size in the 6-<18 years population to be only 0.83 points to achieve the difference of 3 in the overall population of this trial).

Supportive study

Study **B12302** was a Phase IIIb open-label, single arm, multi-center safety study conducted in 27 SMA patients aged 2 to <18 years that discontinued treatment with nusinersen or risdiplam. This may be considered representative of a (potential) large part of anticipated use of OAV101B in the real-word, i.e. in pre-treated patients.

Included patients had 2 or 3 *SMN2* copies, clinical onset <18 months of age or diagnosis via newborn screening, and were able to sit but not to walk independently. Mean age of symptoms onset was 0.78 years (range 0.08-1.42 years), corresponding to (originally) patients with either type 1 (onset ≤6 months of age) or type 2 (onset >6 months) SMA.

Study B12302 primarily investigated safety and tolerability of OAV101B in pretreated patients over 52 weeks. Efficacy measures (change from baseline to Week 52 in HFMSE total score and in RULM total score) were assessed as secondary endpoints.

Long term follow-up studies

Participants who received OAV101B in clinical study B12301, B12302, or CL-102 were offered the possibility to enroll into a long-term follow-up (LTFU) study, and specifically:

- Participants from studies B12301 and B12302 could enroll into LTFU study **A12308**, conducted for 5 years post-dosing, with primary objective safety, and secondary objective efficacy;
- Participants from Study CL-102 could enroll into LTFU study **LT**-**002**, conducted for 15 years post-dosing, with co-primary objectives efficacy and safety.

BBoth studies are currently ongoing.

Efficacy data and additional analyses

Dose finding supportive study - Study **CL-102**

In the 6 to <24 months group (n=13), the primary EP was not met: the motor milestone 'stand alone' was achieved by 1/13 patients, with no difference with natural history data. Exploratory analysis of changes in Bayley-III gross and fine motor skills after 12 months, and of HFSME scores in those patients who reached 2 years of age, were also not clearly supportive of efficacy in this age group: gross motor scores remained substantially stable except for three patients, including the one who also achieved the primary endpoint and may have driven the group results. Based on natural history of SMA type 2/3, small children are more likely to show motor improvement even as "outliers", before declining (Mercuri et al., 2016). For the small subgroup of patients (n=6) who were tested twice with HFMSE, improvement was relevant after age 2 (HFMSE change from baseline =6.7). However, their mean baseline HFMSE score was lower (10.5) than in the 2-5 years old group before treatment (14.8), hence not supportive of efficacy *before* age 2.

In the 24 to <60 months age group (n=12), the primary EP 'change from baseline in HFMSE total score' was met. Despite limitations of an open-label study design (possible observer bias) and the comparison with external controls, the observed improvement in HFMSE score after one year (LSM change from baseline =6.0, 95% CI: 3.7- 8.3) is considered relevant, as exceeds what can be expected from the natural history of non-ambulant SMA patients in this age range (+ .04 points in 12-months, Mercuri et al., 2016).

Therefore, although limited, data from study CL-102 are supportive of efficacy of the proposed therapeutic dose in patients SMA type 2 aged 2 to 5 years. The provided primary, secondary and exploratory data are all *not* supportive of efficacy in patients SMA type 2 aged less than 2 years. Moreover, efficacy cannot be readily extrapolated from patients >2 years to patients aged 6 months to 2 years. The disease phenotype (SMA type 2) was comparable between study CL-102 and study B12301, where efficacy was demonstrated across the study population (2 to <18 years), and appeared to be 'modulated' by age (the younger the patient, the higher the absolute improvement, see discussion of study B12301). In light of this observation, a clearer improvement was expected in patients younger than 2 years in study CL-102 (single arm), which however was observed only in 3 patients out of 13.

As additional justification for the proposed fixed dose, the Applicant conducted a literature search on the CSF volume in paediatric subjects. Based on the provided data, the CSF volume increases within the first 1 to 2 years after birth, after which growth slows and volume remains steady into adolescence. In subjects aged 6 months to 2 years, the CSF increases between 1.0- to 1.6-fold. Based on this, the exposure in paediatric patients aged 6 months to <2 years old may be a factor 1.6-fold higher compared to patients aged ≥2 years. This higher exposure in children below 2 years was not tested in the non-clinical studies, as the pivotal NHP study was conducted in juvenile

monkeys (13-19 months old) corresponding to >3 years of human age (see NC AR). Based on the data in this patient group (n=13), the safety profile appears to be comparable with the older children (see safety discussion). This indicates that the higher exposure might be not clinically relevant, but, due to the limited data provided, no robust conclusions could be drawn.

In conclusion, the totality of clinical and nonclinical data provides support for the proposed posology in patients type 2 SMA aged 2-5 years. Given the stable CSF and spinal cord volume in older children, the proposed posology is considered adequate in the age range 2-18 years, assessed in the pivotal study.

Patients aged 6 months-2 years were included in study CL-102 only (n=13). The totality of the provided clinical and nonclinical data, and the additional Applicant's discussion, was considered insufficient to establish the adequacy of the proposed dose and efficacy in this age range. The inclusion of patients aged 6 months-2 years, as in the originally proposed indication, was therefore not supported, and the target population in section 4.1 of the SmPC was amended to "patients 2 years of age and older". Based on the SmPC section 5.1 guideline (EMA/CHMP/566497/2023), this section was also adequately amended to reflect inconclusive CL-102 study data in the younger age-group.

Pivotal study - Study **B12301**

Of the 217 participants screened, 77/217 (35%) did not meet the inclusion/exclusion criteria. Most exclusions were due to elevated anti-AAV9 antibody titers (27/217, 12%); other reasons were infections (14/217, 6%), or severe contractures/scoliosis (14/217, 6%). None of the participants was excluded due to clinically significant sensory abnormalities. This suggests that sensory neuropathy issues may be not usually encountered in untreated patients in clinical practice.

A total of 126 SMA patients were randomized, 75 in the OAV101B group, and 51 in the Sham group.

Demographic characteristics were overall balanced between treatment groups. The mean age at dosing was 5.88 years (range 2.1-16.6 years). Patients of different races were included, mostly Asians (58.7%). In the context of an European application, this is considered acceptable, as the clinical picture of SMA is not expected to vary across races, and probably an inevitable constraint given by the current differences in patient access to other therapies across geographic areas. Overall, the demographic characteristics of the pivotal study can be considered representative of the amended target population (2 years and older).

The provided baseline disease characterization was unclear at the time the data was submitted for MA. Patients were described to be able to sit independently but never had the ability to walk independently. Mean age at SMA symptom onset, as recalled by caregivers, was 12.7 months, ranging from 6 to 33 months. However, typically developing children acquire the ability to walk independently by approximately 18 months of age. Therefore, it was unclear how patients could have symptom onset as up to 33 months although they were not able to walk independently yet (motor delay is a key symptom of SMA). Moreover, SMA type was not collected in study B12301.

Based on age at onset, study participants would be most likely classified as either type 2 (onset <18 months), who never achieved ambulation, or type 3a (onset >18-36 months), who achieved and then lost ambulation. Subgroup analyses were provided by the Applicant accordingly. However, the Applicant confirmed that none of the patients enrolled in study B12301 were reported to ever have achieved independent walking, which is not compatible with SMA type 3, but consistent with type 2.

The Applicant acknowledged that age at onset as collected by caregivers reports is susceptible to recall bias. Indeed, caregiver reports *alone* are not considered a reliable source of information particularly when age at symptom onset is concerned, as caregivers may have not have sufficient

knowledge to distinguish between normal developmental variations and early signs of motor decline. Particularly as this data was collected retrospectively, and memory can be imprecise in recalling the time of initial, often subtle, neuromuscular signs and symptoms. Therefore, disease history should not rely *solely* on family reports, but at least complemented by medical reports, which are essential to more accurately define symptom onset in clinical studies. As the utilized method for collecting age at onset is not reliable, the provided age at onset data may be not accurate. Indeed, upon revision of the individual patients data, some of the presented combinations of 'age at onset' and 'highest motor function achieved' resulted to be clearly implausible, as discussed above.

In sum, age at symptom onset data were not recorded reliably in this study and are prone to recall bias because of caregiver reporting. Although some cases were identified where the mismatch between the recorded age of symptom onset and the highest motor function achieved was clearly apparent, this raises concerns regarding *all* age at onsets reported by caregivers in this study. In the absence of reliable age at symptom onset data for all subjects, this issue could not be addressed methodologically (e.g. replacing clearly incorrect ages of symptom onset using imputation methods would require the availability of at least some age at symptom onset data that are reliably recorded and medically confirmed/adjudicated). As a consequence, the baseline disease characterization of the study population as provided by the Applicant remains unclear, and age at onset, and any derived measure such as disease duration (age at dosing minus age at symptom onset), cannot be trusted as such. Consequently, the validity of any subgroup analyses based on these variables, as well as any extrapolation of treatment effects, is highly questioned with the provided data.

SMA type was not collected in study B12301. However, based on the provided highest motor function ever achieved (considered reliable as less susceptible of recall bias), the study population is considered to consist only of patients with **SMA type 2**, in line with the study title.

Overall, the disease characteristics of the pivotal study population are not representative of the proposed target population, as treatment naïve patients with other SMA types (type 1, type 3, and type 4) were *not* included. Hence, the inclusion of these patients in the proposed therapeutic indication is not supported by the data.

This particularly applies to **SMA type 1**, which is the most severe, complex (visceral involvement), and rapidly progressive form of the disease in the youngest -i.e., most vulnerable- patients. The Applicant explained that treatment naive patients type 1 were not included in the development program of OAV101B as these patients would be diagnosed shortly after birth and receive SMN-targeted treatment immediately, hence would be considered treatment experienced patients: this is understood and agreed. N=6 treatment-experienced patients who originally fit into the SMA type 1 classification based on their age at onset (≤6 months of age) were enrolled into supportive study B12302. Besides the limited sample size, these patients cannot be considered representative of the treatment-naive SMA 1 population as, at the time of OAV101B injection, patients were able to sit, which clearly deviates from the natural history of type 1 (never sit). The historical SMA classification is less relevant for patients receiving disease modifying treatment, hence the efficacy of OAV101B cannot be extrapolated nor just assumed in these most severe, rapidly progressive, and vulnerable type 1 patients. Moreover, type 1 patients would be diagnosed shortly after birth and should receive SMN-targeted treatment immediately, which does not fit even the originally proposed '6 months and older' target population. The suitability of the IT formulation per se is also questioned in type 1 given visceral involvement, and the reduced systemic distribution of the product. In sum, given the absence of data, impossibility to extrapolate, and unsuitability of the product, the indication of OAV101B needs to **exclude** patients **treatment-naïve SMA type 1.**

Conversely, extrapolation of the indication to the later onset SMA forms, i.e., **type 3** (ambulatory patients) and **type 4** (rare and mild adult form) is considered justified. As per inclusion criteria, study B12301 participants were able to sit but not to walk at baseline. However, their highest motor function ever achieved ranged from 'sitting' to 'walking with assistance', and their baseline HFMSE scores ranged from 1.0 to 42.5 points, hence patients had a relatively broad range of motor function levels. Subgroup analysis showed that the treatment effect was higher in patients with the highest function achieved, and in patients with the highest baseline HFMSE score. This is considered biologically plausible (better motor function = more motor neurons preserved = more neurons available for effective transduction), and supports the hypothesis that the product could be (even more) efficacious in milder forms of the disease, i.e. type 3 and type 4 SMA. Considerations based on age at onset and treatment effect, which would further inform extrapolation to later onset forms, cannot be made with provided data due to age at onset issues. However, extrapolation of efficacy to later onset forms based on motor function status (a proxy of age at onset) is considered justified and sufficient, and there is no indication (from disease pathogenesis, MoA, and experience with other SMN-targeted therapy) suggesting otherwise.
Therefore, the therapeutic **indication** of OAV101B **can include** patients **type 3 and type 4 SMA.**

In addition, the study did not include any adult participants. Reason provided by the Applicant was that scoliosis and/or contractures, which increase with age in SMA, would have confounded and/or prevented the assessment of HFMSE (primary endpoint), which is understood.

Extrapolation from children to adults might be considered if:

- the mechanism of disease is identical in adults, which it is.

- the mechanism of action of OAV101B is age-independent, which may be biologically plausible.

- but disease progression, treatment response, and motor unit reserve may differ significantly between children and adults with SMA. Patients above 18 years of age with a similar functional status as patients included in the pivotal study are expected to benefit from the treatment in a similar way. However, all SMA patients progressively lose function over time. Subgroup analyses in study B12301 based on baseline HFMSE score showed that also in patients with the lowest baseline scores (ranging from 1 to 15) there was a positive treatment effect (LSM difference in HFMSE change from baseline = 1.69), and above the 1.46 MCID value for SMA type 2, which is the most severe phenotype in the proposed indication (being type 1 excluded).
Moreover, even if ambulation is lost, maintenance of gross and fine motor skills of the upper body is of relevance for older patients, to preserve self-autonomy. Patients and caregivers surveys show that any loss of scores, even a single point, is clinically meaningful, and even stabilization is considered a therapeutic progress in SMA (Pera et al., 2017; Rouault et al., 2017).
In light of these considerations, **extrapolation** to **adult** patients is also considered **justified**. The lack of information in this patient population has been adequately reflected in the SmPC.
Together, the provided data and argumentations are considered adequate and sufficient to support efficacy in adult and paediatric patients with SMA type 3 and type 4 (in addition to type 2), from the age of 2 years. The provided justification is considered *not* sufficient to support efficacy in patients with SMA type 1 and in patients aged 6 months to 2 years in general, hence these patients have been excluded (when the efficacy results of the study population cannot be extrapolated to the subgroup in question, a restriction of the indication applies,EMA/CHMP/483022/2019).
By restricting the indication to patients 2 years of age and older, treatment-naïve type 1 patients would be then automatically excluded due to natural history disease course, hence no specification of 'treatable' SMA types is needed. The therapeutic indication was therefore amended as follows:

"Itvisma is indicated for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the *SMN1* gene in patients from 2 years of age and older".

According to current clinical practice in the EU/EEA, Zolgensma is administered to patients below 13 kg of weight. The amended indication of Itvisma partially overlaps with the use of Zolgensma, although to a more limited extent than originally applied (i.e. only for those patients ≥ 2 years but ≤13 kg), providing more clarity for prescribers, who can decide based on the feasibility/suitability of the type of injection per se (IT or IV) in case the patient is eligible for both treatments.

Genetically, most participants of study B12301 had bi-allelic deletion of *SMN1* (122/126, 96.8%), and 4 participants (3.2%) had hemizygous deletions with pathogenic variant in the other chromosome: these proportions are completely in line with literature (e.g., Wirth, Hum Mutat., 2000), hence well representative of the SMA population. Most participants had 3 copies of *SMN2* (119/126, 94.4%), the remaining having either 2 (5/126, 4%) or ≥4 copies (2/126, 1.6%), reported as such as the *SMN2* assay cannot distinguish between 4 copies or more. There were no patients with 1 copy of *SMN2,* as this genotype strongly associates with SMA type 1. Considerations on extrapolation to specific *SMN2* copy numbers in isolation are of relevance when the phenotype (SMA type) is unknown (still), i.e. in asymptomatic patients. Being SMA type 1 only excluded, which can be diagnosed clinically before the age of 6 months, recommendations in asymptomatic patients based on specific genotypes are not required.

The primary endpoint (EP) of study B12301 was change from baseline in HFMSE total score at Week 52, to be tested in the overall population (2 to <18 years, α=0.01) or in patients aged 2-<5 years (α=0.04). The primary EP was met in the overall population: the least square mean (LSM) change from baseline was 2.39 for OAV101B, and 0.51 points for Sham, with a treatment difference of 1.88 (p-value=0.0074). The clinical relevance of the observed, relatively modest, effect (1.88 points) is uncertain but accepted. The effect was not significant in patients aged 2-<5 years.

Secondary analyses in the overall study population, including HFMSE ≥3-point responders and change from baseline in RULM, did not reached statistical significance based on the prespecified testing strategy. However, they may be considered overall supportive of efficacy of OAV101B over Sham.

Secondary and exploratory analyses (change in HFMSE total score, HFMSE ≥3-point responders, and change in RULM total score) were then conducted in two age groups: 2 to <5 years age and 5 to 18 years. Based on previous clinical studies with risdiplam and nusinersen (Mercuri et al., 2018; 2022), greater improvement in the younger patients was expected. These age-group analyses did not reach statistical significance based on the testing strategy. Results were overall suggestive of better efficacy of OAV101B in the older children (5 to <18 years) than in the younger children (2 to <5 years): LSM difference in HFMSE change from baseline was 1.44 in the 2 to <5 years group (95% CI: -0.33 to 3.22; p-value 0.1097), and 2.45 in the 5 to <18 years group (95% CI: 0.42 to 4.49; p-value 0.0193); age group analysis in the HFMSE ≥3-point responders, and change in RULM total score yielded consistent results.

The longitudinal natural history study of Mercuri and colleagues (2019) showed that the rate of progression in SMA type 2 (corresponding to the B12301 study population) is not linear, but follows a curvilinear trajectory with a peak at approximately 5 years of age. This suggests that there is an overall trend to improve until 5 years of age, followed by a steep deterioration until approximately 13 years of age, and then relative stabilization (Mercuri et al., 2019). This is considered a plausible biological explanation of the observed larger treatment effect after age 5 (where there is deterioration) than in children aged 2 to <5 years (where there is natural improvement, which is

reflected in the sham arm of the provided subgroup analysis).

Yet, the same considerations on disease trajectory apply to previous studies where greater improvement in younger patients was found (Mercuri et al., 2018; 2022). This may relate to other differences between the clinical studies (CHERISH for nusinersen or SUNFISH for risdiplam vs B12301), such as patient's disease duration before treatment. Disease duration in B12301 was calculated based on unreliable age at onset data, hence meaningful comparisons cannot be made, and this issue was no further pursued.

The progressively larger absolute improvement in HFMSE in the younger children (OAV101B 2 to <5 years at week 52 = 3.03, at week 64 = 3.46) compared to the older children (OAV101B 5 to <18 years at week 52 = 1.58, at week 64 = 1.91), and the progressive irreversible neuronal loss in SMA, justify treatment with OAV101B from early age.

Treatment response may differ in patients with different disease severity, which correlates with age at onset. There was a signal for a positive interaction between treatment and the highest motor milestone achieved, with the largest treatment effect in patients that achieved "walk with assistance" and the lowest effect in patients that achieved "sitting". Together, these results suggest better efficacy in patients with later onset/milder symptomatology. This suggests greater responsiveness in less severely affected patients, likely due to more preserved motor neurons. Additional subgroup analyses confirmed this hypothesis. HFMSE sensitivity may also vary: it may better detect gains in stronger patients, while underestimating meaningful improvements in weaker ones.

Post-hoc treatment by age interaction analysis confirmed previous findings of larger treatment effect in the older children (5 to <18 years) compared to the younger children (2 to <5 years). However, since study B12301 included a functionally heterogeneous patient population, results may be confounded by disease severity. Additional subgroup analysis in six strata based on highest motor function achieved ('sitting', 'stands', 'walks with assistance') and age group at dosing (2 -<5 years age and 5-18 years) confirmed the notion that these two variables behave as effect modifiers (the higher the function and/or the older the patient > the higher the effect), explaining inconsistencies across the study population.
Overall, these data raise the hypothesis that age is not a relevant variable for outcome in this clinical setting. The unexpected observation that older children (aged >5 years) demonstrated better clinical outcomes than younger ones (aged 2–5 years) indicates that other variables may play a more critical role in determining therapeutic efficacy. In this clinical setting the time between disease symptom onset and treatment seems to be a more relevant variable**.** The scatter plots with LOESS curves clearly illustrate a possible numeric relationship between the variables and the treatment outcome. The use of confidence intervals helps gauge the certainty of the trend lines. Data could have been be presented better by explicitly plotting the two age groups using different colours within the same plot.

The Applicant interprets the data trends, noting that while earlier treatment (with either treatment or placebo) tends to yield slightly better improvement, a "clinically meaningful benefit is maintained regardless of when treatment is initiated," arguing for broad applicability. The figures should be interpreted with caution, as the figure will heavily depend on the LOESS parameters, e.g. a smaller smoothing span would likely result in a different, more variable curve. Although the current data presentation does not raise new signals, conclusive statements on the interaction between treatment and disease duration cannot be made, also given concerns about the reliability of this data.
The vast majority (94.4%) of patients in the pivotal study had 3 *SMN2* copies, hence meaningful subgroup analyses based on different genotypes could not be performed.

Additional post-hoc analysis was conducted using a 1.5 point threshold for HFMSE improvement in the overall study population, and in the 2 to <5 years and 5 to <18 years age groups. This analysis was informed by Coratti et al. (2024), which established a Minimal Clinically Important Difference (MCID) value of 1.46 for improvement in HFMSE in patients with type 2 SMA by using an anchor-based methodology with FDA guidance. This analysis showed that the proportion of participants achieving ≥1.5-point increase in HFMSE total score from baseline to the end of Period 1 was numerically higher in the OAV101B arm than the Sham arm in the overall population, as well as in the 2 to <5 years and 5 to <18 years age groups. Importantly, the Applicant indicated that SMA type was not recorded in the trial. So, what is left is just observations of "positive trends", as the applicant formulates, much less than the expected statistically significant improvements in MCID.

Pooled analyses

Pooled efficacy analyses were conducted in 87 participants from study B12301 (n=75) and CL-102 (n=12 aged 2-5 years who received the therapeutic dose). Interpretation of these analyses is challenging due to methodological differences between the two studies (RCT *vs* open-label). Comparison of results in similar patient groups (2-5 years of age) is informative in this respect:

In the 2 to <5 years group treated with OAV101B in study B12301 (n=42), the LSM change from baseline in HFMSE total score was 3.00 (95% CI: 1.86 - 4.13);
In the 2 to <5 years group treated with OAV101B therapeutic dose in study CL-102 (n=12), the LSM change from baseline in HFMSE total score was 6.0 (95% CI: 3.7 to 8.3).

Both studies suggest improvement after treatment, but of different magnitude. The larger improvement in study CL-102 may be explained by the small sample size, lack of internal comparator and open-label study design, as the HFSME assessment is susceptible of observer bias. Hence, formal comparisons or pooled analysis between B12301 and CL-102 are not considered to be meaningful.

Supportive study - Study **B12302**

Main efficacy results of study B12302 were:

The HFMSE mean change from baseline to Week 52 was 0.17 (range -5.5 to 7.5), and the LSM change from baseline to Week 52 was 1.05 (95% CI: -0.21 to 2.32).
The RULM mean change from baseline to Week 52 was 0.29 (range -6.0 to 6.0), and the LSM change from baseline to Week 52 was 0.59 (95% CI: -0.56 to 1.73).

Results were overall suggestive of stabilization of motor function at 52 weeks, on average. This is particularly relevant in the current therapeutic context within the EU. OAV101B clearly has the advantage over the other therapies of being a one-time treatment. Results may be somewhat confounded by previous treatment, as the effect of OAV101B cannot be completely disentangled from possible long-lasting effects of nusinersen or risdiplam. However, being the half-life of nusinersen in the CSF up to 177 days (25 weeks), and the wash-out period of risdiplam 15 days (2 weeks), their residual effect at 52 weeks is expected to be minimal. It is therefore more plausible that the observed stabilization at the end of the study is due to OAV101B rather than to prior treatment.

The small sample size, as well as the open label study design and lack of comparative arm, hamper the interpretability and generalizability of these results, hence no firm conclusions can be made based on this data.

Moreover, among previously treated patients with nusinersen or risdiplam, there could be patients not able to pursue the treatment albeit responding (hence who may consider OAV101B for convenience), and patients non responding to the treatment for whom OAV101B could represent a rescue therapy. As both types of patients were included in study B12302, data were stratified accordingly. The Applicant informed that 6 out of 27 (22%) study participants discontinued prior treatment due to reported lack of efficacy. Most of these patients (5 out of 6) showed a substantially stable HFMSE trajectory up to 52 weeks, while one patient deteriorated, similarly to patients that discontinued prior treatment due to other reasons. Therefore, there was no clear distinction between the two groups. This suggests that OAV101B may be effective (considering stabilization as a therapeutic progress) also in patients non responding to other SMN-targeted therapies. However, the limited sample size, and the lack of HFMSE data before OAV101B treatment, hamper the interpretability of these study results.

Long-term follow-up studies

In the first round, at the time of data cut-off for submission for MA, 6 months FU data were available for 13/59 patients from study A2308, and approximately 4 years FU data from 12 patients were available for study LT-002. Concomitant treatment with nusinersen or risdiplam was allowed in both LTFU studies, and used by all patients in study LT-002. In the second round, the Applicant provided extended follow up data with a more recent data cut-off (for study A12308: 24-Jun-2025 = additional 7.5 months data; for study LT-002: 30-Jun-2025 = additional 12 months data). In these studies, additional therapies have been used by 15 out of 91 patients (16%) in study A12308, and by 9 out of 12 (75%) patients in study LT-002.

Overall, these data are supportive of long term efficacy, as mostly showing either stabilization or further improvement over a time span of approximately 2 years for study A12308, and 4-5 years for study LT-002. This was confirmed by long term efficacy data of Zolgensma, where concomitant treatments were also allowed.

Furthermore, the Applicant provided additional 3 months of follow up data from study B12301, which showed continued and progressive motor function improvement in those 3 months, in the absence of additional therapies.

On the one hand, the provided long term follow-up data are of relatively limited duration (in the context of a life-long disease), and complicated by concomitant use of disease-modifying therapies. Both nusinersen and risdiplam target *SMN2*, and therefore contribute to raise the SMN protein level via an additive, but possibly also synergistic, complementary mechanism. Interpretation of long-term efficacy of OAV101B alone is therefore hampered, although it may be assumed that it was not such as to disregard additional treatment. This indicates that, although OAV101B has the potentiality to eliminate long-term burden of chronic therapies, in practice these are used nevertheless.

On the other hand, the provided data do not clearly show a differential pattern between patients that used Itvisma/Zolgensma in isolation and patients that employed add-on treatments. Moreover, concomitant therapy is probably unavoidable, especially in the long run, and is representative of the likely real-world scenario where patients make use of all available (if suitable) treatment options. Which is expected, given the severe progressive nature of the disease, and the different MoA of nusinersen/risdiplam *vs* Itvisma/Zolgensma.

Overall, and despite the intrinsic limitations of the performed long-term follow-up studies, the provided data are considered sufficient to support maintenance of efficacy for the MAA of Itvisma, in the current scenario where other therapeutic options are available. In line with the Zolgensma SmPC, a concise description of the available longer term follow-up data (study LT-002) has been added to section 5.1.

Patient / caregiver engagement
For a more comprehensive characterization of the impact of SMA and treatments, the Applicant conducted interviews with caregivers of patients as an exploratory objective of study B12302 (treatment-experienced patients).
Due to the intrinsic limitations of this study (qualitative semi-structured data and limited sample size, N=22), no conclusions can be drawn from this data on the impact of the treatment with OAV101B. However, this study provides insights into the quality of life of caregivers and patients pre and post-treatment, which is considered valuable.

GCP
A routine GCP inspection was conducted by the EMA in the South-Africa site (Cape Town), Vietnam site (Hanoi), and Sponsor site (Basel, Switzerland). Compliance with GCP and applicable regulations were verified, with focus on the verification of selected efficacy and safety data reported in the MAA.

The EMA inspection of the sponsor site identified 4 critical findings.

Overall, the reported critical findings were considered serious, and in some cases structural, inadequacies of several aspects of the clinical trial conduct. The Applicant was asked to address all the reported critical and major findings with adequate corrective and preventive actions (CAPA). The provided CAPA were considered generally adequate by the EMA inspection team. The EMA inspection team concluded that the reported findings did affect, to some extent, the quality of the data. In addition, as a result of the number of major and critical findings on relevant GCP aspects, the GCP-compliance was considered impacted. However, the inspection team also concluded that, from the GCP perspective, the (core) trial results can be used in this procedure.

In conclusion, it is concurred with the EMA inspection team that the core trial results can be used in this MA procedure, and it is agreed with the Applicant that the interpretation of the main study results remains unchanged. Although the GCP compliance of the study was affected, this likely did not impact the usability of the core study dat.

5.3.9.2. Conclusions on the clinical efficacy

The Applicant submitted the data of a sufficiently sizable, randomized, double-blind, sham-controlled pivotal trial (B12301), demonstrating efficacy of OAV101B on clinically relevant aspects of the disease in a restricted (SMA type 2) non-ambulatory but able to sit independently patient population, aged 2 to <18 years. The primary endpoint, change in HFMSE total score, showed a statistically significant improvement compared to sham control, with consistent numerical advantages observed across all secondary efficacy measures, although these did not reach statistical significance under the hierarchical testing strategy. Subgroup analyses showed that older children (5 to <18 years) had greater and more consistent motor gains than younger children (2 to <5 years), reflecting differences in disease progression across the life span.

The impact of effect modifiers such as disease severity and age on treatment response has been investigated by subgroup analysis, and is considered elucidated. Despite the relatively short duration and concomitant use of disease-modifying treatments in the provided LTFU studies, long-term data are considered sufficient to support maintenance of efficacy for MA.

The Applicant was asked to provide additional justification for the indication of OAV101B in patients beyond the pivotal study population for age, i.e. in patients aged 6 months- <2 years, and ≥18 years.

The clinical program of OAV101B was conducted in patients with SMA type 2, hence the Applicant was asked to discuss extrapolation to patients with other disease phenotypes.

The provided data and argumentations are considered supportive of efficacy in adult and paediatric patients with SMA type 3 and type 4 (in addition to type 2), from the age of 2 years. The provided justification is considered not sufficient to support extrapolation of efficacy (and safety) to patients with SMA type 1 or between 6 months and 2 years of age in general, hence the Applicant amended the indication as requested to:

"… for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene in patients 2 years of age and older."

Critical GCP findings were identified at the Sponsor site, but likely had no impact on the core study data, hence on the assessment of the B/R.

5.4. Clinical safety

5.4.1. Safety data collection

The clinical safety of OAV101B has been evaluated in 5 studies. Safety assessments included adverse events (AEs), serious adverse events (SAEs) and adverse events of special interest (AESI), laboratory assessment, vital signs, physical examinations including neurological examination, and cardiac evaluations (ECG and echocardiogram) collected at study visits.

Safety data from Study B12301 sham-controlled 52-week Period 1 form the basis for the primary safety assessment of OAV101B in participants with SMA.

Two different analyses of pooled safety data provide integrated safety evidence:

- The 52-week pooled safety analysis (N=127) was based on pooled safety data from all participants who received the OAV101B 1.2x1014 vg dose in Study B12301 Period 1 (n=75), Study B12302 (n=27, and Study CL-102 (n=25). This analysis provides supportive safety data for OAV101B 1.2x1014 vg dose with up to 52 weeks of follow-up in both treatment naïve (studies B12301 and CL-102) and previously treated (Study B12302) participants with SMA aged 6 months and older.

- The Long-term safety analysis (N=101) was based on pooled safety data from all participants who received the OAV101B 1.2x1014 vg dose (in studies B12301 (Period 1), B12302, or CL-102) and were followed-up beyond 52 weeks (in Study B12301 Period 2, LT-002, or A12308). The long-term safety analysis focused on SAEs and AESI reported from the end of the Week 52 period and up to data cut-off of the individual studies.

The MedDRA version used for the pooled analyses was 27.1.

5.4.2. Patient exposure

A total of 167 participants have been exposed to OAV101B at the target dose.

Table 20: Patient exposure (cut off)

	Patients enrolled	Patients exposed*	Patients exposed to the proposed dose range	Patients with long term** safety data
Blinded studies (placebo-controlled)	126 (treated in sham-controlled Period 1 of B12301)	75	75	Long-term follow-up is described below.
Blinded studies (active -controlled)	0	0	0	0
Open studies	59 (27 in B12302, 32 in CL-102)	59	52 (27 in B12302, 25 in CL-102)	Long-term follow-up is described below.
Post marketing	0	0	0	0
Compassionate use	0	0	0	0

* Received at least 1 dose of active treatment

** In general this refers to 6 months and 12 months continuous exposure data, or intermittent exposure

Overall follow-up time since OAV101B dosing is described in Table 21 below.

Table 21: Overview of follow-up time (years) since OAV101B dosing (pooled safety analysis set)

Study	Cohort/Arm	N	Mean (SD)	Median (min-max)
COAV101B12301 (B12301)	OAV101-SHAM arm (Dosed with OAV101B in Period 1)	75	1.28 (0.103)	1.25 (1.0 - 1.6)
	SHAM-OAV101 arm (Dosed with OAV101B in Period 2)	46	0.24 (0.048)	0.23 (0.2 - 0.5)
COAV101B12302 (B12302)	Overall	27	0.97 (0.148)	1.00 (0.3 - 1.1)
AVXS-101-CL-102 (CL-102)	Cohort 2 (Dose B): Age 6 to <24 months	13	1.00 (0.017)	1.00 (1.0 - 1.0)
	Cohort 2 (Dose B): Age 24 to <60 months	12	0.99 (0.024)	0.99 (1.0 - 1.1)
	Cohort 2 (Dose B): Overall	25	0.99 (0.020)	1.00 (1.0 - 1.1)
AVXS-101-LT-002 (LT-002)	Cohort 2 (Dose B): Age 6 to <24 months	8	5.50 (1.622)	5.78 (2.9 - 7.2)
	Cohort 2 (Dose B): Age 24 to <60 months	4	6.28 (1.235)	6.88 (4.4 - 6.9)
	Cohort 2 (Dose B): Overall	12	5.76 (1.496)	6.78 (2.9 - 7.2)
COAV101A12308 (A12308)	STEER	71	1.83 (0.612)	1.92 (0.6 - 2.9)
	STRENGTH	20	1.94 (0.257)	1.89 (1.6 - 2.3)
	Overall IT	91	1.86 (0.554)	1.91 (0.6 - 2.9)

Source: **Error! Bookmark not defined.**

5.4.3. Adverse events

Table 22: Summary of adverse events (full analysis set)

	Study B12301 Period 1		52-week pooled safety analysis
	OAV101B N=75	**Sham N=51**	**OAV101B N=127**
Category	**n (%)**	**n (%)**	**n (%)**
Any AE	73 (97.3)	46 (90.2)	125 (98.4)
Any AE related to study treatment	26 (34.7)	6 (11.8)	50 (39.4)
Any SAE	21 (28.0)	17 (33.3)	31 (24.4)
Any SAE related to study treatment	8 (10.7)	1 (2.0)	9 (7.1)
Any severe AE	8 (10.7)	9 (17.6)	17 (13.4)
Any AE leading to study discontinuation	1 (1.3)	1 (2.0)	1 (0.8)
Any AE leading to death	0	0	0

For each category, patients are included only once, even if they experienced multiple events in that category.

The 52-week pooled safety analysis included participants who received OAV101B $1.2x10^{14}$ vg in Study B12301 Period 1, Study B12302, and Study CL-102.

Source: SCS-Table 2-1

Study B12301 Period 1

Adverse events reported in more than 5% of participants in either treatment arm of Study B12301 Period 1 are shown in Table 23 below.

Table 23: Treatment-emergent adverse events (>5% of participants in either treatment arm) in study B12301 period 1 (safety analysis set)

	OAV101B N=75	**Sham N=51**
Preferred term	**n (%)**	**n (%)**
Number of participants with at least one event	73 (97.3)	46 (90.2)
Upper respiratory tract infection	26 (34.7)	15 (29.4)
Pyrexia	19 (25.3)	12 (23.5)
Vomiting	15 (20.0)	6 (11.8)
Constipation	11 (14.7)	11 (21.6)
Cough	11 (14.7)	11 (21.6)
Pneumonia	9 (12.0)	8 (15.7)
Headache	8 (10.7)	2 (3.9)
Lower respiratory tract infection	8 (10.7)	6 (11.8)
Nasopharyngitis	7 (9.3)	5 (9.8)
Influenza	5 (6.7)	5 (9.8)
Nausea	5 (6.7)	3 (5.9)
Oropharyngeal pain	5 (6.7)	0
Dizziness	4 (5.3)	1 (2.0)
Pharyngitis	3 (4.0)	4 (7.8)
Respiratory tract infection	3 (4.0)	4 (7.8)

Preferred term	OAV101B N=75 n (%)	Sham N=51 n (%)
Bronchitis	3 (4.0)	3 (5.9)
Rash	2 (2.7)	3 (5.9)
Respiratory tract inflammation	2 (2.7)	3 (5.9)
Urticaria	2 (2.7)	4 (7.8)
Diarrhoea	1 (1.3)	8 (15.7)

n=Number of participants;
A participant with multiple occurrences of an AE for a preferred term is counted only once in each specific category.
MedDRA Version 27.1.
Source: Study B12301 52w-Table 14.3.1-3

To evaluate adverse events related to the gene therapy procedure as a whole, treatment-emergent adverse events that were reported within 72 hours of treatment during study B12301 Period 1 are summarized in the Table 24 below.

Table 24: Treatment-emergent adverse events reported within 72 hours of treatment with study treatment by preferred term in study B12301 period 1 (safety analysis set)

Preferred term	OAV101 1.2e14 vg N=75 n (%)	Sham N=51 n (%)
Number of patients with at least one event	29 (38.7)	12 (23.5)
Vomiting	11 (14.7)	1 (2.0)
Headache	5 (6.7)	1 (2.0)
Nausea	5 (6.7)	1 (2.0)
Pyrexia	4 (5.3)	3 (5.9)
Constipation	3 (4.0)	0
Dizziness	3 (4.0)	1 (2.0)
Abdominal pain	2 (2.7)	0
Back pain	2 (2.7)	0
Decreased appetite	2 (2.7)	0
Viral infection	2 (2.7)	0
Urticaria	1 (1.3)	2 (3.9)

n=Number of patients.
Preferred terms are sorted by descending frequency of OAV101 column.

Only events occurring in more than 2 participants in one arm are presented.
A patient with multiple occurrences of an AE for a preferred term is counted only once in each specific category

MedDRA Version 27.1.
If time is not available for an adverse event, all adverse events occurring on Day 1-4 are included.

[Source: Table 1-2.haq-eu1]

52 week pooled safety set

Adverse events reported in more than 5% of participants in the 52-week pooled analysis are shown in Table 25 below.

Table 25: Treatment-emergent adverse events (>5% of participants) in 52-week pooled safety analysis (52 week pooled safety set)

Preferred term	**Overall N=127 n (%)**
Number of patients with at least one event	125 (98.4)
Pyrexia	46 (36.2)
Upper respiratory tract infection	44 (34.6)
Vomiting	36 (28.3)
Nasopharyngitis	27 (21.3)
Cough	26 (20.5)
Constipation	18 (14.2)
Headache	17 (13.4)
Pneumonia	12 (9.4)
Pain in extremity	10 (7.9)
Influenza	9 (7.1)
Abdominal pain upper	8 (6.3)
Lower respiratory tract infection	8 (6.3)
Nausea	8 (6.3)
Oropharyngeal pain	8 (6.3)
Rash	8 (6.3)
Rhinorrhoea	8 (6.3)
Abdominal pain	7 (5.5)
Back pain	7 (5.5)
COVID-19	7 (5.5)
Nasal congestion	7 (5.5)
Scoliosis	7 (5.5)

52-week pooled safety set (52WPSS) includes all participants that received OAV101B 1.2e14 vg during their primary parent study core period (study CL-102 whole period, B12301 Period 1 only, and B12302 whole study period).

n = Number of participants with adverse events during the primary phase of the parent studies.

A participant with multiple occurrences of an AE for a preferred term is counted only once in each specific category.

MedDRA Version 27.1

Source: SCS Appendix 1-Table 2-2.1.2

5.4.3.1. Adverse drug reactions

Table 26: Summary of ADRs proposed for inclusion by the applicant in the SmPC

Infections and infestations	
Very Common	Upper respiratory tract infection[a)]
Blood and lymphatic system disorders	
Common	Thrombocytopenia
Nervous system disorders	
Very common	Headache[b)]
Common	Dizziness[b)]
Common	Peripheral sensory neuropathy[c)]
Common	Hypoaesthesia
Common	Paraesthesia
Gastrointestinal disorders	
Very common	Vomiting
General disorders and administration site conditions	
Very common	Pyrexia
Investigations	
Common	Hepatic enzyme increased[d)]

a) Upper respiratory tract infection includes upper respiratory tract infection, viral upper respiratory tract infection, rhinitis, oropharyngeal pain and viral infection.
b) Adverse reactions considered related to the lumbar puncture procedure.
c) Peripheral sensory neuropathy includes peripheral sensory neuropathy and neuropathy peripheral.
d) Hepatic enzyme increased includes hepatic enzyme increased, alanine aminotransferase increased, aspartate aminotransferase increased, hepatitis, hypertransaminasaemia and hepatic function abnormal.

* Source: SCS Appendix 1-Table 2-3.2.1, Table 2-3.2.2

The ADRs presented in the table above are based on 127 treatment-naïve and treatment-experienced participants with SMA who received OAV101B at the recommended dose (1.2x1014 vg) in Study B12301 Period 1, Study B12302, and Study CL-102. The age at time of OAV101B administration ranged from 0.6 to 17.7 year, and the duration of follow-up was 52 weeks.

ADRs are classified according to MedDRA system organ classification and frequency. Frequency categories are derived according to the following conventions: very common (⩾1/10); common (⩾1/100 to <1/10); uncommon (⩾1/1 000 to <1/100); rare (⩾1/10 000 to <1/1 000); very rare (<1/10 000); not known (cannot be estimated from the available data). Within each frequency grouping, adverse reactions are presented in order of decreasing seriousness.

5.4.4. Adverse events of special interest, serious adverse events and deaths, other significant events

5.4.4.1. Adverse events of special interest

The adverse events of special interest (AESI) associated with onasemnogene abeparvovec treatment were identified based on the clinical and non-clinical experience, and correspond to the important identified and important potential risks identified for the OAV101 IV and IT programs. The AESI are:

- Hepatotoxicity

- Transient thrombocytopenia
- Cardiac adverse events
- Signs and symptoms that may be suggestive of dorsal root ganglia (DRG) toxicity
- Thrombotic microangiopathy
- New malignancien

In the long-term safety analysis, 'new incidence of neurological, autoimmune and hematological disorders' were defined as AESI to capture potential AEs in long term follow-up studies after administration of human gene therapy products. Broad search criteria were used to retrieve events, with the aim to increase the likelihood to capture AEs that may be related to OAV101.

AESI were summarized and listed based on Standardized MedDRA terminology. The MedDRA terms used to define the AESIs are specified in the program eCRS.

An overview of AESI categories reported in Study B12301 Period 1 and in the 52-week pooled safety analysis is provided in Table 27 below.

Table 27: Overview of treatment-emergent adverse events of special interest in study B12301 period 1 and the 52-week pooled safety analysis

	Study B12301 Period 1		**52-week pooled safety analysis**
	OAV101B N=75	**Sham N=51**	**OAV101B N=127**
AESI	**n (%)**	**n (%)**	**n (%)**
Hepatotoxicity	7 (9.3)	5 (9.8)	16 (12.6)
Transient thrombocytopenia	4 (5.3)	2 (3.9)	16 (12.6)
Signs and symptoms that may be suggestive of DRG toxicity	2 (2.7)	1 (2.0)	4 (3.1)
Cardiac adverse events	0	0	1 (0.8)
New malignancies	0	0	1 (0.8)
Thrombotic microangiopathy	0	0	0

The 52-week pooled safety analysis included participants who received OAV101B $1.2x10^{14}$ vg in Study B12301 Period 1, Study B12302, and Study CL-102.

Source: SCS-Table 2-11

Hepatotoxicity AESI

To mitigate the risks associated with immune response to the AAV capsid, all patients are given immunomodulatory therapy with prednisolone starting the day before OAV101 administration, and continuing for up to 2 months or until resolution of any transaminase elevations observed. Hepatotoxicity AESI reported in Study B12301 Period 1 and in the 52-week pooled safety analysis are summarized in Table 28 below.

Table 28: Treatment-emergent hepatotoxicity AESI reported in study B12301 period 1, and the 52-week pooled analysis

	Study B12301 Period 1		52-week pooled analysis
	OAV101B N=75 n (%)	**Sham N=51 n (%)**	**OAV101B N=127 n (%)**
Any hepatotoxicity AESI	**7 (9.3)**	**5 (9.8)**	**16 (12.6)**
Blood alkaline phosphatase increased	1 (1.3)	0	4 (3.1)
Hepatic enzyme increased	1 (1.3)	1 (2.0)	4 (3.1)
Aspartate aminotransferase increased	1 (1.3)	1 (2.0)	3 (2.4)
Hepatic function abnormal	2 (2.7)	2 (3.9)	2 (1.6)
Hepatitis	2 (2.7)	1 (2.0)	2 (1.6)
Alanine aminotransferase increased	0	2 (3.9)	1 (0.8)
Hypertransaminasaemia	0	0	1 (0.8)
Hepatomegaly	0	0	1 (0.8)
Glutamate dehydrogenase increased	0	1 (2.0)	0

The 52-week pooled safety analysis included participants who received OAV101B $1.2x10^{14}$ vg in Study B12301 Period 1, Study B12302, and Study CL-102.

Source: SCS Table 2-12

Transient thrombocytopenia AESI

Transient thrombocytopenia AESI reported in Study B12301 Period 1 and in the 52-week pooled safety analysis are summarized in Table 29 below.

Table 29: Transient thrombocytopenia treatment emergent AESI through the end of follow-up period 1 by treatment (SAF)

	Study B12301 Period 1		52-week pooled analysis
	OAV101B N=75 n (%)	**Sham N=51 n (%)**	**OAV101B N=127 n (%)**
Any transient thrombocytopenia AESI	4 (5.3)	2 (3.9)	16 (12.6)
Contusion	1 (1.3)	1 (2.0)	5 (3.9)
Epistaxis	0	0	3 (2.4)
Thrombocytopenia	2 (2.7)	0	2 (1.6)
Haemoglobin decreased	1 (1.3)	0	1 (0.8)
Subcutaneous haematoma	0	1 (2.0)	0
Activated partial thromboplastin time prolonged	0	0	1 (0.8)
Bone contusion	0	0	1 (0.8)
Gastric haemorrhage	0	0	1 (0.8)
Haematochezia	0	0	1 (0.8)
Haemoglobin decreased	0	0	1 (0.8)
Lower gastrointestinal haemorrhage	0	0	1 (0.8)
Petechiae	0	0	1 (0.8)

	Study B12301 Period 1		52-week pooled analysis
	OAV101B N=75 n (%)	Sham N=51 n (%)	OAV101B N=127 n (%)

The 52-week pooled safety analysis included participants who received OAV101B $1.2x10^{14}$ vg in Study B12301 Period 1, Study B12302, and Study CL-102.

Source: Study B12301-Table 14.3.1-13, SCS Appendix 1-Table 2-2.7.3

Thrombotic microangiopathy

No cases of TMA were reported in Study B12301 Period 1 or in the 52-week pooled safety analysis.

Cardiac adverse events AESI

No cardiac adverse events AESI were reported in Study B12301 Period 1.

In the 52-week pooled safety analysis, cardiac adverse events AESI were reported in 1 participant (0.8%). This was an event of blood creatine phosphokinase MB increased, reported in a participant in Study CL-102. The event resolved

Signs and symptoms that may be suggestive of DRG toxicity

DRG toxicity is considered an important potential risk associated with OAV101 treatment due to OAV101-related pathologic findings in DRG of cynomolgus monkeys. These findings were asymptomatic and were not associated with nerve conduction study abnormalities. In NHPs receiving the IT equivalent dose, these findings resolved at Week 52 (see also the [Toxicology Written Summary] for details on the non-clinical findings). Similar findings have been reported after administration of AAV vectors in other studies in rhesus macaques and mini pigs.

A meta-analysis by Hordeaux et al (2020) reported that the pathology after AAV vector-administration in NHPs was minimal to moderate in most cases, clinically silent in affected animals, and characterized by mononuclear cell infiltrates, neuronal degeneration, and secondary axonopathy of central and peripheral axons on histopathological analysis. The etiology of DRG inflammation is complex and not well understood.

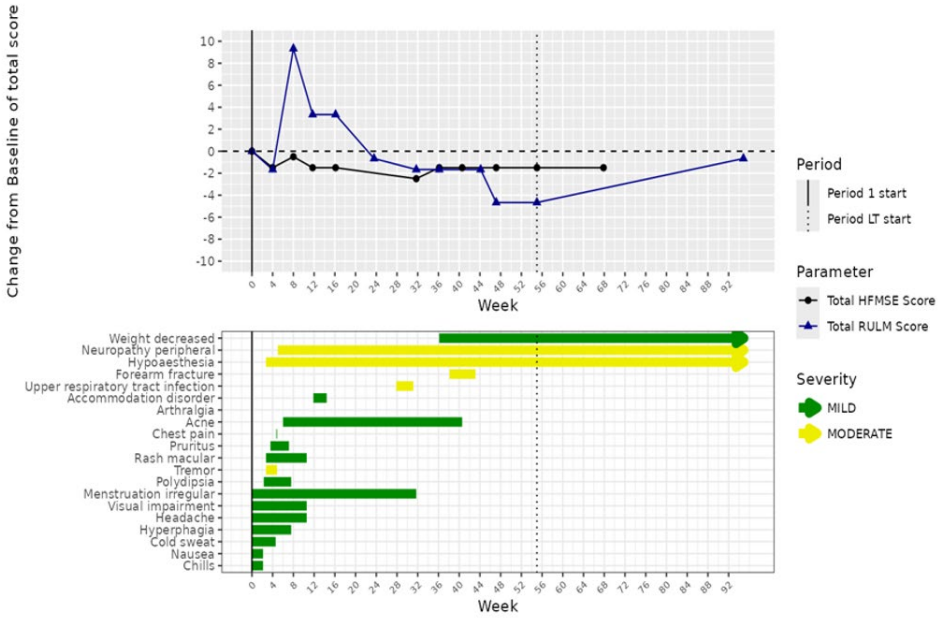
The search criteria for the categorization of adverse events suggestive of DRG toxicities are broad and optimized for sensitivity rather than specificity.

In Study B12301 Period 1, there were 3 participants with treatment-emergent AEs reported under the AESI signs and symptoms that may be suggestive of DRG toxicity: 2 participants (2.7%) in the OAV101B arm, and 1 participant (2.0%) in the Sham arm. There were 2 participants in study B12302 which also reported AESI signs and symptoms suggestive of DRG toxicity. The case narratives for all 5 participants are summarized below[1]:

- **Study B12301 Period 1** :

[1] Summary made by Rapporteur clinical safety assessor based on CSR electronic narratives described in study CSRs B12301 Period 1 and B12302

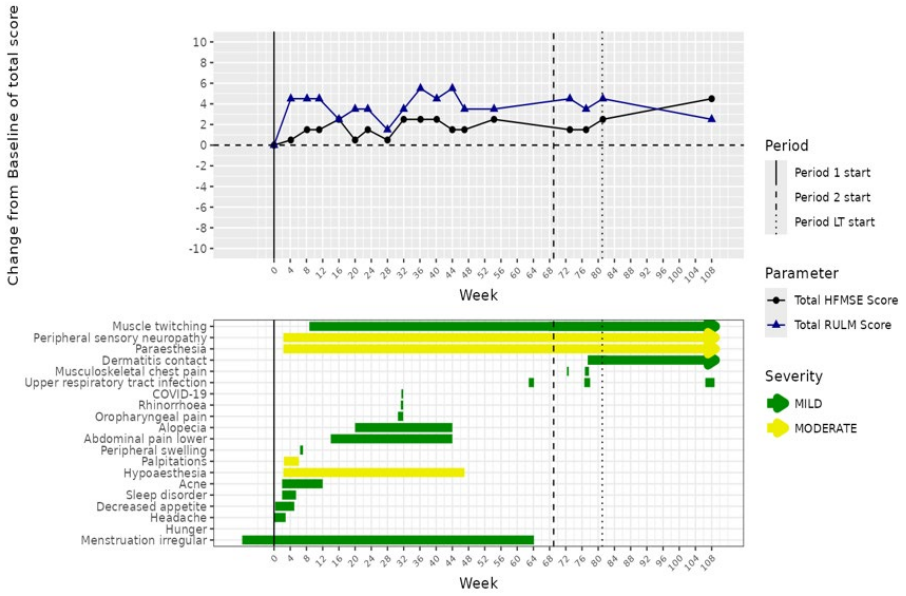
Figure 18: study B12301 (Treatment: period 1 OAV101B/prednisolone, period 2 not eligible), AESI: hypoaesthesia, neuropathy peripheral



On Day 20 in study B12301 after OAV101B administration in Period 1, one participant experienced a serious event of hypoaesthesia, followed by a non-serious event of neuropathy peripheral on Day 36. The participant was not considered eligible to continue to period 2 of the study due to exclusion criteria of "clinically significant sensory abnormalities in the neurological examination at week 48" being met. After discontinuing from Study B12301, the participant enrolled in Study A12308. Both events were reported as ongoing at the time the participant discontinued study B12301. The adverse events were reported in the participant's medical history in the A12308 long-term study as ongoing, without signs of worsening as of the data cut off used for the analysis. Further details on this participant, including the treatment received are presented in section a) of this response. The participant's change from B12301 baseline to Week 52 HFMSE was –1.5, and RULM was –4.7 in study B12301. At the latest assessments available in Study A12308, the severity of the sensory symptoms had lessened since the onset and the change from B12301 Baseline in HFSME was stable at –1.5 (Week 68 post-OAV101 and in RULM had improved almost to baseline at –0.7 (Week 95 post-OAV101).

- **Study B12301 Period 1** :

Figure 19: study B12301 (Treatment: period 1 OAV101B/prednisolone, period 2 sham/placebo), AESI: hypoaesthesia, paraesthesia, peripheral sensory neuropathy



On Day 17 in Study B12301 following OAV101B administration in Period 1, one participant experienced a non-serious event of hypoaesthesia which resolved within 314 days, a non-serious event of paraesthesia and a serious event of peripheral sensory neuropathy, which were ongoing at the time of the completion of the B12301 study. After completing the Study B12301, the participant enrolled in Study A12308. Paraesthesia and peripheral sensory neuropathy were captured in the participant's medical history in the long-term study as ongoing, without signs of worsening as of the data cut off used for the analysis. Further details on this participant, including the treatment received are presented in section (a) of this response. The participant's change from B12301 baseline to Week 52 in HFMSE was +2.5 points, and RULM was +3.5 points. At the latest visit in Study A12308 (Week 108 post-OAV101, 03 June 2025), change from B12301 Baseline in HFSME was +4.5 and in RULM was +2.5.

- **Study B12302 Period 1**: This participant was randomized to the Sham arm in Period 1 and received OAV101B in Period 2 in study B12301. 154 days following Sham administration in Period 1, the participant experienced a non-serious mild event of paraesthesia (reported term: 'the enveloping sensation of the left thigh spreading all over the body') which resolved within 15 days. On Day 20 in B12301 Period 2 following OAV101B administration, the participant experienced an episode of paraesthesia (reported term: 'sensation of double plantar foreign body during sleep' which resolved within 2 days. Both events were mild in nature and did not require treatment. After completing Study B12301, the participant enrolled in Study A12308. At the participant's latest visit in A12308 (Week 71 post-OAV101), change from B12301 Week 52 (1 day prior to OAV101 administration) in HFSME was +5 and RULM was -2.

- In study B12302 there were also two narratives of potential DRG toxicity. On Day 28 in Study B12302 following OAV101B administration in Study B12302, a participant experienced a non-serious mild event of paraesthesia (reported term: 'different sensation in feet – tingling'). Neurological examination was normal at this time and throughout the study, and assessment of sensory nerve action potentials was not conducted. Around the same time on Day 30, the participant demonstrated a new ability to stand unsupported for more than a count of 3 (score 2 on item #19 on HFMSE, prior scores 0). The adverse event of paraesthesia resolved after 63 days without treatment.

38 days following OAV101B administration in Study B12302, another participant experienced three non-serious mild events reported as sensory disturbance (reported terms: sensory change in bilateral forearms, sensory change in neck and sensory change in scalp). Neurological examination was normal at this time and throughout the study, and assessment of sensory nerve action potentials was not conducted. The sensory symptoms in bilateral forearms resolved the following day (Day 39) without any treatment, and the sensory symptoms in neck and scalp resolved the day after that (Day 40), also without any treatment. The participant's change from baseline to Week 52 in HFMSE score was 1, and RULM was 2. Hence, the participant continues to demonstrate progress in motor function, and there is no impact from the sensory adverse events. After completing the Study B12302, the participant chose not to enroll into Study A12308.

New malignancies (including risk of tumorigenicity as result of vector integration)

No new malignancy AESI were reported in Study B12301 Period 1. In the 52-week pooled safety analysis, the search retrieved one new malignancy AESI in 1 participant (0.8%). This was an AE of acrochordon (skin tag) reported in Study CL-102; this event was not considered related to study treatment and the event was ongoing at the end of the study.

No new malignancy AESI were reported in the long-term follow-up studies LT-002 and A12308.

Recently, the FDA placed a clinical hold on its investigational gene therapy RGX-111 (source: clintrials.gov), for the treatment of MPS I (Hurler syndrome), following preliminary analysis of a single case of neoplasm (intraventricular CNS tumor) in a participant treated in its Phase I/II study. The case was identified during a routine brain MRI of an asymptomatic participant who received intracisternal RGX-111. Preliminary genetic analysis of the resected tumor detected an AAV vector genome integration event associated with overexpression of a proto-oncogene (PLAG1), which is known to be susceptible to chromosomal rearrangements. For this specific case, the analysis is still ongoing and causality has not yet been established.

New incidence of neurological disorders

In the long term safety set there were 4 participant reported AEs reported in this AESI category. There were 3 events of headache (3%) and 2 of dizziness (2%).

5.4.4.2. Serious adverse events and deaths

In Study B12301 Period 1, SAEs (regardless of study drug relationship) were reported in 21 participants (28.0%) in the OAV101B arm and 17 participants (33.3%) in the Sham arm. By SOC, the most common SAEs by SOC were infections and infestations (20.0% vs. 33.3%) and gastrointestinal disorders (6.7% vs. 2.0%) (Study B12301 52w-Table 14.3.1-6).

SAEs reported in Study B12301 Period 1 and the 52-week pooled analysis are summarized by PT in Table 30 and Table 31 below.

Table 30: Treatment-emergent serious adverse events reported in at least 2 participants in either arm in study B12301 period 1 (safety analysis set)

Preferred term	OAV101B N=75 n (%)	Sham N=51 n (%)
Number of participants with at least one event	21 (28.0)	17 (33.3)
Pneumonia	9 (12.0)	7 (13.7)
Vomiting	3 (4.0)	0
Influenza	2 (2.7)	0
Lower respiratory tract infection	2 (2.7)	4 (7.8)
Pneumonia viral	1 (1.3)	2 (3.9)
Pneumonia bacterial	0	2 (3.9)

MedDRA Version 27.1.
Source: Study B12301 52w-Table 14.3.1-7

SAEs reported in the 52-week pooled analysis are summarized by PT in Table 31 below.

Table 31: Treatment-emergent serious adverse events by preferred term (in at least 2 participants) (52-week pooled safety set)

Preferred term	Overall N=127 n (%)
Number of patients with at least one event	31 (24.4)
Pneumonia	11 (8.7)
Influenza	3 (2.4)
Vomiting	3 (2.4)
Headache	2 (1.6)
Lower respiratory tract infection	2 (1.6)
Pyrexia	2 (1.6)

52-week pooled safety set includes all participants that received OAV101B 1.2e14 vg during their primary parent study core period (study CL-102 whole period, B12301 Period 1 only, and B12302 whole study period).
MedDRA Version 27.1
Source: SCS Appendix 1-Table 2-2.5.2

In the long-term safety analysis, 17/101 participants (16.8%) experienced SAEs after 52 weeks or more of follow-up post OAV101B dosing. The most frequent SAEs by SOC were infections and infestations (14.9%). The most frequently reported SAE by PT was pneumonia (7.9%).

No deaths occurred in participants treated with OAV101B in studies B12301, B12302, or CL-102 (any dose cohort), or in the long-term follow-up studies LT-002 or A12308.

5.4.5. Discontinuation due to adverse events

Discontinuing study treatment is not possible, as OAV101B is given as a single IT administration.

Two participants (one in each treatment arm) were reported as having discontinued from Study B12301 due to AE. The participant in the OAV101B arm completed Period 1, but did not meet eligibility criteria

for Period 2 and was therefore discontinued from the study. An AE of peripheral neuropathy was erroneously reported as having led to discontinuation from the study. The participant in the Sham arm discontinued due to a severe SAE of pneumonia aspiration that was considered recovered/resolved by the end of Period 1.

5.4.6. Safety in special populations

OAV101B has been studied in paediatric patients ⩾6 months of age only. No information is available in the elderly.

No studies have been conducted in patients with hepatic or renal impairment.

No studies have been performed on the effects of OAV101B in pregnant and lactating women. It is not known whether OAV101B has the potential to be transferred to the fetus. Therefore, a thorough benefit-risk evaluation is needed prior to treatment of women who are pregnant or desire to become pregnant.

Use of OAV101B across the age cohorts

Subgroup analyses based on age cohort were provided for the 52-week pooled analyses. An overview of AE categories by age and overall reported in the 52-week pooled safety analysis is provided intables below. No significant difference in the nature or type of adverse events has been identified across age groups.

Table 32: Overview of treatment-emergent adverse events by age group and overall (52-week pooled safety set)

	≥6 months to <2 years	**≥2 to <5 years**	**≥5 to <18 years**	**Overall**
Category	**N=13 n (%)**	**N=60 n (%)**	**N=54 n (%)**	**N=127 n (%)**
Any treatment-emergent adverse event	13 (100.0)	60 (100.0)	53 (98.1)	126 (99.2)
Any treatment-emergent adverse event related to study treatment	7 (53.8)	23 (38.3)	21 (38.9)	51 (40.2)
Any serious treatment-emergent adverse event	2 (15.4)	17 (28.3)	12 (22.2)	31 (24.4)
Any serious treatment-emergent adverse event related to study treatment	1 (7.7)	4 (6.7)	4 (7.4)	9 (7.1)
Any severe treatment-emergent adverse event	4 (30.8)	12 (20.0)	1 (1.9)	17 (13.4)
Any treatment-emergent adverse event leading to study discontinuation	0	0	0	0
Any treatment-emergent adverse event leading to death	0	0	0	0
Any treatment-emergent adverse event of special interest	6 (46.2)	12 (20.0)	15 (27.8)	33 (26.0)
Hepatotoxicity	4 (30.8)	7 (11.7)	5 (9.3)	16 (12.6)
Transient thrombocytopenia	4 (30.8)	5 (8.3)	7 (13.0)	16 (12.6)
Thrombotic microangiopathy	0	0	0	0
Cardiac adverse events	0	1 (1.7)	0	1 (0.8)
Signs and symptoms that may be suggestive of dorsal root ganglia toxicity	0	1 (1.7)	3 (5.6)	4 (3.1)
New malignancies	0	1 (1.7)	0	1 (0.8)

Numbers (n) represent counts of participants with adverse events during the primary phase of the parent studies.
52-week pooled safety set (52WPSS) includes all participants that received OAV101B 1.2e14 vg during their primary parent study core period (study CL-102 whole
period, B12301 Period 1 only, and B12302 whole study period).
Adverse events of special interest according to OAV101 (CRS ID 210252) Compound Case Retrieval Strategy definition for indication Spinal Muscular Atrophy active from 2025-10-30.
Source: [COAV101B1 - EU HAQ2- Table 2-1.c.haq-eu2]

Table 33: Treatment-emergent adverse events by preferred term (in >10% of participants overall) by age group and overall (52 week pooled safety set)

	≥6 months to <2 years	**≥2 to <5 years**	**≥5 to <18 years**	**Overall**
Preferred term	**N=13 n (%)**	**N=60 n (%)**	**N=54 n (%)**	**N=127 n (%)**
Number of patients with at least one event	13 (100.0)	60 (100.0)	53 (98.1)	126 (99.2)
Pyrexia	6 (46.2)	24 (40.0)	16 (29.6)	46 (36.2)
Upper respiratory tract infection	10 (76.9)	20 (33.3)	14 (25.9)	44 (34.6)
Vomiting	5 (38.5)	16 (26.7)	15 (27.8)	36 (28.3)
Nasopharyngitis	3 (23.1)	11 (18.3)	14 (25.9)	28 (22.0)
Cough	3 (23.1)	13 (21.7)	11 (20.4)	27 (21.3)
Constipation	2 (15.4)	13 (21.7)	3 (5.6)	18 (14.2)
Headache	0	2 (3.3)	15 (27.8)	17 (13.4)

52-week pooled safety set (52WPSS) includes all participants that received OAV101B 1.2e14 vg during their primary parent study core period (study CL-102 whole period, B12301 Period 1 only, and B12302 whole study period).
n = Number of participants with adverse events during the primary phase of the parent studies.
A participant with multiple occurrences of an AE for a preferred term is counted only once in each specific category.
Preferred terms are sorted in descending frequency of overall column.
MedDRA Version 28.1
Source: [COAV101B1 - EU HAQ2- Table 2-2.1.2.a.haq-eu2]

Table 34: Treatment-emergent serious adverse events by preferred term (in at least 2 participants overall) by age group and overall (52 week pooled safety set)

	>=6 months to <2 years	**>=2 to <5 years**	**>=5 to <18 years**	**Overall**
Preferred term	**N=13 n (%)**	**N=60 n (%)**	**N=54 n (%)**	**N=127 n (%)**
Number of patients with at least one event	2 (15.4)	17 (28.3)	12 (22.2)	31 (24.4)
Pneumonia	0	9 (15.0)	2 (3.7)	11 (8.7)
Influenza	0	2 (3.3)	1 (1.9)	3 (2.4)
Vomiting	0	3 (5.0)	0	3 (2.4)
Headache	0	0	2 (3.7)	2 (1.6)
Lower respiratory tract infection	0	2 (3.3)	0	2 (1.6)
Pyrexia	0	0	2 (3.7)	2 (1.6)

52-week pooled safety set (52WPSS) includes all participants that received OAV101B 1.2e14 vg during their primary parent study core period (study CL-102 whole period, B12301 Period 1 only, and B12302 whole study period).
n = Number of participants with adverse events during the primary phase of the parent studies.
A participant with multiple occurrences of an AE for a preferred term is counted only once in each specific category.
Preferred terms are sorted in descending frequency of overall column.
MedDRA Version 28.1.
Source: [COAV101B1 - EU HAQ2- Table 2-2.5.2.a.haq-eu2]

5.4.7. Immunological events

The baseline serum anti-AAV9 antibody titers were below 1:50 in all OAV101B treated participants. Increases from baseline in anti-AAV9 antibody titers were observed in all OAV101B treated participants and remained elevated at 12 months following OAV101B administration with median titers ⩾1:819200 in Phase III studies (Study B12301, Study B12302). Baseline CSF anti-AAV9 antibodies were negative for all participants. There was no clear association of post-dose serum anti-AAV9 titers with safety.

No anti-SMN antibody response was observed in any participant in Phase I/II dose-ranging study (Study CL-102) measured by ELISA. Anti-SMN antibody responses in Phase III studies were measured by ECLIA which is generally more sensitive than ELISA. Positive pre-existing anti-SMN antibodies were observed in one participant in Study B12302. By the clinical database cut-off date of December 2024, within the 12-month period after OAV101B administration, positive anti-SMN antibodies were observed in 5/75 (6.7%) participants and 2/27 (7.4%) participants at one or more timepoints in Study B12301 and Study B12302, respectively. The observed antibody titers ranged from 1:10 to 1:80.

5.4.8. Safety related to drug-drug interactions and other interactions

Not applicable

5.4.9. Vital signs and laboratory findings

Haematology parameters

In Study B12301 Period 1, a transient decrease in platelet count was observed in the first week after dosing in the OAV101B arm. Mean platelet count decreased from 326.9x109/L at baseline to 273.3x109/L at Week 1 (mean change from baseline was -53.3x109/L) and returned thereafter towards baseline levels (figure below, Study B12301 52w-Table 14.3-4.1.1).

Shifts to grade 1 decrease (i.e. <LLN to 75x109/L) in platelet count were observed in 45.3% vs. 23.5% of participants in the OAV101B arm vs the Sham arm, respectively. Grade 3 decreases (i.e. <50x109/L to 25x109/L) were noted in 2.7% vs 2.0% of participants, and grade 4 (i.e. <25x109/L) were noted in 1.5% and 2.0% of participants in the OAV101B arm and Sham arm, respectively (Study B12301 52w-Table 12-10). The final post baseline platelet values were grade 1 in 9.3% vs. 3.9% of participants in the OAV101B arm vs. the Sham arm, and none of the participants had a final platelet value that was grade 2 or higher.

Biochemistry parameters

In Study B12301 Period 1, increases in transaminases (ALT or AST >3x ULN) were observed in 5.3% vs. 5.9% of participants, and ALT or AST >5x ULN was observed in 4.0% vs. 2.0% of participants. Increases in transaminases >8x ULN were only observed in the OAV101B arm (4.0%). None of the participants had increases in total bilirubin >1.5x ULN, and none of the participants met the biochemical criteria for Hy's Law (see table below).

Overall, most of the participants experienced mild and transient transaminase elevations which occurred approximately between weeks 6 and 12. No clinically meaningful changes in troponin I, alkaline phosphatase, or bilirubin were seen through the end of Period 1 in either treatment arm (Study B12301 52w-Figure 14.3-1.3).

In the 52-week pooled analyses, ALT or AST >3x ULN were observed in 4.7% of participants. ALT or AST >5x ULN and >8x ULN were observed in 3.9% of participants. None of the participants had increases in total bilirubin >1.5x ULN, and none of the participants met the biochemical criteria for Hy's Law.

Table 35: Summary of participants meeting elevation criteria for liver function test based on laboratory data in study B12301 period 1, and the 52-week pooled safety analysis

	Study B12301		**52-week pooled safety set**
Elevation criteria	**OAV101B N=75 n (%)**	**Sham N=51 n (%)**	**Overall N=127 n (%)**
ALT > 2 x ULN	13 (17.3)	5 (9.8)	18 (14.2)
ALT > 3 x ULN	4 (5.3)	3 (5.9)	6 (4.7)
ALT > 5 x ULN	3 (4.0)	1 (2.0)	5 (3.9)
ALT > 8 x ULN	3 (4.0)	0	5 (3.9)
ALT > 10 x ULN	3 (4.0)	0	4 (3.1)
ALT > 20 x ULN	2 (2.7)	0	2 (1.6)
AST > 2 x ULN	3 (4.0)	1 (2.0)	7 (5.5)
AST > 3 x ULN	3 (4.0)	0	5 (3.9)
AST > 5 x ULN	2 (2.7)	0	2 (1.6)
AST > 8 x ULN	1 (1.3)	0	1 (0.8)
AST > 10 x ULN	1 (1.3)	0	1 (0.8)
AST > 20 x ULN	0	0	0
ALT or AST > 2 x ULN	13 (17.3)	5 (9.8)	19 (15.0)
ALT or AST > 3 x ULN	4 (5.3)	3 (5.9)	6 (4.7)
ALT or AST > 5 x ULN	3 (4.0)	1 (2.0)	5 (3.9)
ALT or AST > 8 x ULN	3 (4.0)	0	5 (3.9)
ALT or AST > 10 x ULN	3 (4.0)	0	4 (3.1)
ALT or AST > 20 x ULN	2 (2.7)	0	2 (1.6)
Total bilirubin > 1.5 x ULN	0	0	0
Total bilirubin > 2 x ULN	0	0	0
Total bilirubin > 3 x ULN	0	0	0
ALT or AST > 3 x ULN and Total bilirubin > 2 x ULN *	0	0	0
ALT or AST > 3 x ULN and Total bilirubin > 2 x ULN and ALP >= 2 x ULN *	0	0	0
ALT or AST > 3 x ULN and Total bilirubin > 2 x ULN and ALP <2 x ULN *	0	0	0

ULN= upper limit of normal, ALP= alkaline phosphatase, ALT= alanine aminotransferase, AST= aspartate aminotransferase.
n = Number of patients meeting elevation criteria at least once at any post-baseline visit.
Only post-baseline results meeting elevation criteria are included.
*For the combination categories, the values do not need to have been collected at the same assessment, it can be any post baseline value.

52-week pooled safety set (52WPSS) includes all participants that received OAV101B 1.2e14 vg during their primary parent study core period (study CL-102 whole period, B12301 Period 1 only, and B12302 whole study period).

Source: Study B12301 52w-Table 14.3-4.3.1, SCS Appendix 1-Table 4-1.3

Vital signs

No clinically meaningful differences were observed between arms in study B12301 period 1.

In the 52-week pooled analysis, the most common vital sign abnormalities were high diastolic blood

pressure (97.6%), high systolic blood pressure (96.1%) and high pulse rate (91.3%). These changes were transient and returned towards baseline values at the final observations at Week 52.

Electrocardiograms

In Study B12301 Period 1, QTcF values that were >30 ms and ⩽ 60 ms prolonged from baseline were observed in 20.0% vs. 27.5% of participants in the OAV101B vs. Sham arms, respectively. No QTcF values that were >60 ms prolonged from baseline, or were >450 ms, were observed in either arm. No abnormalities in ECG results were reported as AEs.

In the 52-week pooled safety analysis, QTcF values that were >30 ms to ⩽ 60 ms prolonged from baseline were observed in 20.5% of participants. Similar to Study B12301 Period 1, there were no QTcF values that were >60 ms prolonged from baseline, or were >450 ms.

Echocardiogram

In Study B12301, no difference between arms was observed for echocardiogram results, these were in line with the troponin results.

In the 52-week pooled safety analysis, there were 2 additional participants who met the criteria for low cardiac function, i.e. LVEF <56% or LVFS <28%

5.4.10. Overall discussion and conclusions on clinical safety

5.4.10.1. Overall assessment of available safety data

Safety of Itvisma/OAV101B was evaluated in 5 clinical studies, of which the primary source of safety evaluation is the 52-week randomized, double-blind, sham-controlled study B12301 Period 1. In addition, safety has been pooled into a 52-week pooled analysis which includes all studies at the 52-week mark (B12301 Period 1, B12302 and CL-102) and the long-term safety analysis which has safety data > 52 weeks (B12301 Period 2 and ongoing long term studies LT-002, A12308). The proposed pooling strategy is agreed as these all evaluated the same dose of OAV101B ($1.2x10^{14}$ vg). In the long-term safety analysis, only serious adverse events (SAEs) and adverse events of special interests (AESIs) are recorded.

Exposure

A total of 167 participants have been exposed to OAV101B, of which all received the target dose. While the size of the overall safety database appears acceptable, safety data did not allow for a full assessment of safety in all relevant subgroups (e.g. age, SMA type).

The overall duration of follow-up varies between the (ongoing) studies. As of the data cut-off dates of the individual studies, the overall median follow-up time since OAV101B dosing in parent studies, in the Pooled Safety Analysis Set was 17.22 months (min-max: 2.6 – 86.7 months) and the median age at data cut off was 6.77 years (min-max: 2.0 – 18.8 years).

Adverse drug reactions

OAV101B is in essence the same gene therapy as Zolgensma (Onasemnogene abeparvovec, EMEA/H/C/004750), albeit a different dose and route of administration (intrathecal instead of intravenous). Therefore the assessment of the ADR profile of OAV101B is focused on differences in comparison with the established ADR profile for intravenous Onasemnogene abeparvovec. The Applicant proposes 11 ADRs in section 4.8 of the Itvisma SmPC.

Pyrexia and **vomiting** have been previously identified with Zolgensma treatment and are also agreed

as ADRs for Itvisma given high frequency of reporting after OAV101B treatment (25.3% and 20.0% respectively). **Headache** and **dizziness** are new ADRs and occurred within the first 72 hours after treatment. It is considered likely that these events are due to the IT route of administration, and these events are also attributed as such in the ADR table in section 4.8 of the SmPC.

Upper respiratory tract infection (including oropharyngeal pain, viral infection, viral upper respiratory tract infection and rhinitis) was reported as an ADR and it was the most frequently reported AE for both treatment arms (34.6% and 29.4% respectively). Causality with OAV101B treatment has been confirmed.

Thrombocytopenia and **hepatic enzyme increased** are known ADRs associated with Zolgensma. Overall, AESIs related to transient thrombocytopenia/thrombotic microangiopathy (12.6% in the 52-week pooled analysis) and hepatic enzyme increased/hepatoxicity (ALT or AST >3x ULN: 4.7% in 52-weel pooled analysis) were mild and transient for most participants. These data suggest that OAV101B may be less thrombotic and hepatotoxic than Zolgensma and it is considered that this is likely due to less systemic exposure after IT administration.

ADRs **peripheral sensory neuropathy**, **hypoesthesia** and **paraesthesia** are suggestive of dorsal root ganglia (DRG) toxicity. Non-clinical findings have indicated pathological findings in the DRG and in humans, DRG injury and inflammation is typically referred to as sensory neuropathy which has been included as an AESI in the clinical development program. Nerve conduction measured by sural and radial sensory nerve action potential (SNAP) was included to complete the neurological examination in study B12301, however it was not structurally measured throughout the study; at baseline and afterwards only in the event of sensory abnormalities. Three cases of peripheral sensory neuropathy were identified in the clinical development program (2 in study 12301 Period 1 and 1 in study B12302) which were attributed to OAV101B treatment. Persistent reductions in SNAP amplitudes were seen the lower extremities of two participants (of the pivotal study), of which in one progression to the upper extremities was observed. These two cases are persistent, i.e., at the last visit of study the events were not resolved and the participants still received treatment for symptoms (e.g. gabapentin). The AESI has not been resolved in both cases. Both participants continue to have symptoms despite ongoing treatment in the long term extension studies. Additional information has been provided with respect to OAV101B efficacy in these participants. One participant appears to have deteriorated on the HFMSE and RULM. Although from the recent cut-off there appears to be some improvement, efficacy is less pronounced compared to another participant who continued to have motor function improvement despite symptom onset. Based on these two cases, the impact of peripheral sensory neuropathy on OAV101B efficacy cannot be determined.

Given the persistent nature of the two narratives from study B12301 Period 1, the risk for peripheral sensory neuropathy following OAV101B cannot be excluded. Additional discussion has been provided on (early) signs and symptoms, treatment and early identification strategies. With respect to the two persistent cases there does not appear to be a pattern or a specific sequence of events. Based on the totality of information available on the two persistent cases of neuropathy it is agreed with the Applicant that there are no risk factors or any additional identification strategies that could be included in the SmPC. There is no clear pattern in terms of signs and symptoms that would be informative for HCP's and caregivers to pay particular attention to. SNAP measurement does not appear to be predictive for the sensory neuropathy, given that there have been patients with symptoms and normal SNAP and vice versa. Based on the available data the warning as currently incorporated in section 4.4 of the SmPC is sufficient.

Section 4.8 of the SmPC includes available information of the two persistent cases still experiencing moderate symptoms despite multiple rounds of additional treatment.

Pain in extremity was proposed as a common ADR, but this has not been identified as a common AE

in the corresponding safety analysis (see Table 14 and Table 20). This ADR is of interest in the light of the identified DRG toxicity, as DRGs are important for pain sensation in the extremities. This is however best covered by the ADR peripheral sensory neuropathy, which includes pain associated with a neuropathic condition, e.g. nerve damage. Further, upon evaluation the "pain in extremity" cases this ADR was not included in section 4.8 of the Itvisma SmPC, as no pattern or common factor could be determined. Thus, "pain in extremities" does not reflect DRG toxicity best as this term is too general, as it does not imply a specific cause.

All participants in the study received a prophylactic course of prednisolone (OAV101B group) or matching placebo (sham group) of a median duration of 59 days. Overall, prednisolone related AEs were infrequent in the OAV101B group and most are in line with the known safety profile of the drug. Vomiting (n=6, 8.0%) is not a known AE associated with prednisolone, however additional discussion has indicated that vomiting cannot be excluded as an ADR associated with oral prednisolone given the imbalance between OAV101B and sham and that it has been reported previously in literature.

Safety data of special interest (risk of tumorigenicity as result of vector integration)

Recent data emerging from the clinical program of the investigational gene therapy, RGX-111, prompted a reevaluation of the purely theoretical nature of tumorigenicity as result of vector integration. The risk for tumorgenicity due to vector integration into the genome is no longer purely theoritcal but potential. Currently tumor samples are being analysed for vector integration into the genome. While analysis of these samples is awaited, the risk of vector integration can be further monitored in the regular PSURs and no additional monitoring or studies are required.

Serious adverse events and deaths

In pivotal study B12301 Period 1, SAEs were reported with a slightly higher frequency in the sham treated group compared to OAV101B (33.3 and 28.0%, respectively). All SAEs in Study B12301 Period 1 and the 52-week pooled safety analysis are related to (viral) infections and infestations.

No deaths occurred in the studies that investigated OAV101B.

Discontinuation due to adverse events

OAV101B is a single IT administration of a gene therapy hence treatment cannot be discontinued. Two participants in study B12301 period 1 discontinued the study due to an AE (one in each treatment arm). The participant in the sham group discontinued due to SAEs of viral infections at the recommendation of the physician. The participant in the OAV101B group discontinued the study had also reported AESIs indicative of peripheral sensory neuropathy which was not resolved at the last study visit in study B12301 Period 1. The Applicant states that "An AE of peripheral neuropathy was erroneously reported as having led to discontinuation from the study". Although this participant did not discontinue due to the AESI, it was due to abnormal neurological examination and it is assumed this is the persistent decreased SNAP amplitude, thus per protocol was not allowed to continue in the study.

Safety in special populations

The sought indication of Itvisma is "for the treatment of SMA with a bi-allelic mutation in the SMN1 gene in patients 6 months of age and older." In the clinical development program, OAV101B was evaluated in patients aged 6 months to 17.7 years with SMA type 2 and 3a. At the moment, the interpretation of the safety database for the full indication is hampered as information is lacking with respect to safety in specific age groups as well as SMA types. Subgroup analyses were only performed for race and gender which in the context of a gene therapy are not very informative. Additional subgroup analyses have been performed across the age cohorts 6 months - < 2 years, 2 years - < 5 years and 5 – < 18 years.

For the 6 months - < 2 years cohort, common treatment-emergent adverse events (> 10%) were pyrexia, upper respiratory tract infection and vomiting. These have also been reported in the older age cohorts albeit with a lower incidence. It is known that young children are more commonly susceptible to (viral) infections. With respect to the other adverse events, unique events for the youngest age group were infrequent and do not suggest any additional risk for this patient group not previously identified. Serious adverse events were infrequent. There was a higher incidence of adverse events of special interest in the youngest age group compared to the older age groups (46.2% vs. 20% and 27.8%, respectively). These were only reported in the category of hepatotoxicity and transient thrombocytopenia, there were no events related to peripheral sensory neuropathy, cardiac adverse events and new malignancies. Closer inspection of the specific AESIs revealed that for hepatotoxicity these were primarily related to increased liver enzyme measurements and for transient thrombocytopenia contusion was most frequently reported. Most of these events have also been reported in the older age groups albeit with lower incidence. As these events are all previously identified as related to the overall gene therapy treatment (including immunosuppressive regimen), there appear to be no additional risks related to the AESIs in the youngest age group. For this age cohort, the available safety data suggests no major differences compared to the older cohort. However, the available data is limited, only 13 patients were treated with the target dose of OAV101B. Concidering the uncertainty regarding the available safety data in in patiens aged 6months to<2 years of age, this population is currently excluded from the indication. No subgroup analyses based on SMA type could be provided as SMA type was not logged in the studies. The provided analyses based on age of SMA onset is not considered reliable as this was not was not recorded reliably in the study and is prone to recall bias because of caregiver reporting.

There is no information available on OAV101B in elderly patients, which is acknowledged given that it is unlikely that SMA is diagnosed in this patient subpopulation.

Non-clinical data indicate no impact on fertility. However, there is no clinical safety data available on the impact of OAV101B on pregnancy and lactation. The absence of data including the precautions are adequately described in section 4.6 of the SmPC.

Immunogenicity

In support of "Use in patients with anti-AAV9 antibody titres > 1:50 and higher vector loads", the applicant referenced a nonhuman primate study. Several issues are identified that hamper a solid conclusion on the clinical impact (see discussion of the safety specs below). As the impact on the efficacy and safety in this population is remains unknown, an appropriate warning is included in section 4.4. of the SmPC.

Laboratory and other findings

Transient decreases in platelet count associated with OAV101B treatment were observed in the first week of the pivotal study and the 52-week pooled analysis, which later returned to baseline levels. Most of these decreases were mild, and none of the participants had a final platelet value that was grade 2 or higher supporting the notion that the decreases in platelet count observed are transient.

Similarly, increases in transaminases (ALT and/or AST) were reported more frequently after OAV101B treatment. Most of the increases observed were mild and transient (between weeks 6 and 12) and none of the participant met the criteria for Hy's law.

With respect to the electrocardiogram and echocardiogram, no clinically relevant findings were reported.

5.4.10.2. Conclusions on clinical safety

The safety profile of Itvisma is largely in line with what has been previously identified for the intravenous formulation of onasemnogene abeparvovec (Zolgensma). Uncertainties remain with respect to potential DRG toxicity given the limited data available (2 persistent cases). Based on the totality of information available on these cases, the warning on DRG toxicity as currently incorporated in section 4.4 of the SmPC is sufficient.

For the 6 months- 2 years of age cohort, the available safety data suggests no major differences compared to the older cohort. However, available data with the target dose of OAV101B is limited. Considering the uncertainty that the available safety data in this cohort may not fully capture the safety profile, the restriction of population is required at this time to allow safe and effective use.

6. Risk management plan

6.1. Safety specification

6.1.1. Proposed safety specification

The applicant proposed the following summary of safety concerns in the RMP:

Table 36: Summary of safety concerns in the proposed RMP

Summary of safety concerns	
Important identified risks	• Hepatotoxicity • Transient thrombocytopenia • Thrombotic microangiopathy
Important potential risks	• Dorsal root ganglia toxicity/peripheral sensory neuropathy • Tumorigenicity due to chromosomal integration
Missing information	• Long-term safety of onasemnogene abeparvovec therapy

The RMP for onasemnogene abeparvovec covers both Zolgensma (onasemnogene abeparvovec administered IV) and OAV101B (onasemnogene abeparvovec administered IT), as both medicinal products contain the same active substance. The list of safety concerns presented in the table above pertain to both Zolgensma and OAV101B but excludes those pertaining to Zolgensma only.

Hepatotoxicity: The administration of an AAV vector has the risk of causing elevated transaminases and immune-mediated hepatotoxicity. A certain capsid load may activate capsid-specific T cells that may lead to hepatotoxicity and loss of transgene expression (Kuranda and Mingozzi 2017). Mild (grade 1) transient increases were observed in participants treated with OAV101B in the clinical trials, without association with clinical signs or symptoms. No cases of liver failure were reported.

In order to mitigate potential for hepatotoxicity, a prophylactic prednisolone regimen was implemented in all OAV101B clinical trials.

Transient thrombocytopenia: Transient thrombocytopenia, manifesting as a transient decrease in platelet count within the first week of dosing, was observed in clinical studies in the OAV101 IV program and in the post-marketing setting for Zolgensma. The mechanism is unknown. In participants treated with OAV101B, a small, transient decrease in platelet count was observed within the first week post OAV101B dosing, with mean values returning to baseline thereafter.

Thrombotic microangiopathy: TMA is a rare, life-threatening condition characterized by thrombocytopenia, microangiopathic haemolytic anaemia, and acute kidney injury. TMA was initially not observed in OAV101 clinical trials but in the post-marketing setting of Zolgensma. No cases of TMA have been reported in participants treated with OAV101B.

Dorsal root ganglia toxicity/peripheral sensory neuropathy: DRG toxicity is considered an important potential risk associated with OAV101 treatment due to OAV101-related pathologic findings in DRG of cynomolgus monkeys. These findings were asymptomatic and were not associated with nerve conduction study abnormalities.

Peripheral sensory neuropathies were reported in 2 OAV101B-treated participants (both in Study B12301), however the non-clinical findings of DRG inflammation have not been confirmed in patients from clinical trials or post-marketing experience.

Tumorigenicity due to chromosomal integration: There is a theoretical risk of tumorigenicity due to integration of AAV vector DNA into the genome. Onasemnogene abeparvovec is composed of a non-replicating AAV9 vector whose DNA persists largely in episomal form. Rare instances of random vector integration into human DNA are possible with recombinant AAV. The clinical relevance of individual integration events is unknown, but it is acknowledged that individual integration events could potentially contribute to a risk of tumorigenicity.

No cases of malignancies have been reported in the OAV101B clinical studies.

Long-term safety of onasemnogene abeparvovec therapy: Follow-up data in SMA patients treated with Itvisma are available from two follow-up studies: LT-002 and COAV101A12308. Study LT-002 enrolled 18 participants with a median follow-up time of 5.32 years; study COAV101A12308 enrolled 59 participants. Therefore, the long-term safety remains Missing Information for Itvitsma, and will be further evaluated as part of the pharmacovigilance plan.

6.1.2. Discussion on proposed safety specification

The presentation of the safety concerns in the RMP is acceptable.

6.2. Pharmacovigilance plan

6.2.1. Proposed pharmacovigilance plan.

Routine pharmacovigilance activities beyond ADRs reporting and signal detection

Specific adverse reaction follow-up checklists:

Specific adverse event follow-up checklists– applicable for Zolgensma or Itvisma - will be used to collect data to further characterize and/or closely monitor each of the respective safety concerns specified below:

- Hepatotoxicity

Onasemnogene abeparvovec elevated liver enzymes checklist

- Transient thrombocytopenia

Onasemnogene abeparvovec decreased platelet count checklist

- Thrombotic microangiopathy

Onasemnogene abeparvovec thrombotic microangiopathy (TMA) checklist

- Cardiac adverse events(applicable for Zolgensma only)

Onasemnogene abeparvovec cardiac adverse events checklist

- **Dorsal root ganglia toxicity**/peripheral sensory neuropathy

Onasemnogene abeparvovec sensory neuropathies checklist

Other forms of routine pharmacovigilance activities for risks

None

Additional pharmacovigilance activities

Clinical studies

Table 37: Ongoing and planned additional pharmacovigilance activities

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorization				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorization or a marketing authorization under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
Study AVXS101LT001 (applicable for Zolgensma only) Ongoing	To collect LTFU safety data of patients with SMA Type 1 who were treated with onasemnogene abeparvovec in the AVXS101CL101 study	• Hepatotoxicity • Thrombotic microangiopathy • Transient thrombocytopenia • Cardiac adverse events • Dorsal root ganglia toxicity/peripheral sensory neuropathy • Tumorigenicity due to chromosomal integration • Long-term efficacy of onasemnogene abeparvovec therapy	Final study report	Q4 2033
Study AVXS101LT002 Ongoing	To collect LTFU safety and efficacy data in patients with SMA Type 1, Type 2 or Type 3 who were treated with onasemnogene abeparvovec in a clinical trial.	• Hepatotoxicity • Thrombotic microangiopathy • Transient thrombocytopenia • Cardiac adverse events • Dorsal root ganglia toxicity/peripheral sensory neuropathy • Tumorigenicity due to chromosomal integration • Long-term safety of onasemnogene abeparvovec therapy	Final study report	Q3 2036
COAV101A12308 (applicable for	To collect LTFU safety and monitoring of	• Hepatotoxicity	Final study	Q3 2031

Itvisma only) **Ongoing**	efficacy in patients with SMA who were treated with OAV101 in clinical trials.	• Transient thrombocytopenia • Thrombotic microangiopathy • Dorsal root ganglia toxicity/peripheral sensory neuropathy • Tumorigenicity due to chromosomal integration • Long-term safety of onasemnogene abeparvovec therapy	report	
SMA Registries Study Feasibility Assessment (applicable for Itvisma only) Planned	To evaluate, in a structured manner, whether data from existing SMA registries is fit for purpose, to evaluate: the incidence, risk factors, clinical course, and outcome of peripheral sensory neuropathy, long-term patient follow-up (up to 15 years) with focus on the risk of tumorigenicity due to chromosomal integration and if effectiveness measures are routinely collected and available in the population not included in the clinical studies (SMA Type 3 or 4).	• Dorsal root ganglia toxicity/Peripheral sensory neuropathy • Tumorigenicity due to chromosomal integration	Submission of a feasibility outcome report Submission of a registry-based study protocol or alternative proposal as post-authorization measure	Within 3 months following the marketing authorisation approval To be confirmed following consultation with EMA

6.2.2. Discussion on the pharmacovigilance plan

6.2.2.1. Routine pharmacovigilance activities

Questionnaires are proposed to be used in line with the safety specification for Zolgensma and/or Itvisma respectively. The proposed follow up questionaires are in line with Zolgensma and thus acceptable. However, in line with Zolgensma, additional pharmacovigilance activities are also required, see below.

6.2.2.2. Additional pharmacovigilance activities

Two long-term follow up studies including patients exposed to Itvisma are currently ongoing; they are included as Category 3 studies in the RMP.

AVXS-101-LT-002 (LT-002) is an ongoing follow up study from patients (**n=18**) who participated in the Phase I/II dose ranging Study CL-102 of IT onasemnogene abeparvovec (and patients treated with Zolgensma in clinical trials). The study duration is up to 15 years from time of dosing in parent study.

The study comprises 2 phases, an initial 5-year FU phase, with visits every 6 months for Years 0 to 2 and annually for Years 3 to 5, followed by an observational phase during which participants are contacted annually via telephone. Co-primary objectives are efficacy and safety. Enrolment is completed.

COAV101A12308 (A12308) includes participants from studies B12301 and B12302 (in addition to patients treated with Zolgensma). A total of **59 patients** who received IT Itvisma are included. The study is comprised of a baseline period and 2 follow-up period. In the first 2 years (follow-up period 1) visits occur every 6 months and thereafter (follow-up period 2) visits are conducted annually. The total duration of FU in study A12308 is 5 years after enrolment in the study. The duration of FU was reduced from 15 years to 5 years after enrolment into the study, with amended protocol version 04, dated 01-Aug-2024. This was acknowledged. The proposed indication of Itvisma includes patients of all ages ≥2 years. Attaining an older age is associated with a milder phenotype. Currently there are no data of safety or maintenance of effectiveness of onasemnogene abeparvovec over a long period of time in patients with anticipated long/normal survival. In older and less severe disabled patients, DRG toxicity with persisting severe neuropathy may even affect the B/R of Itvisma in this subgroup of patients. Therefore, long term follow up is needed to characterise the risk of DRG toxicity concerning incidence, potential risk factors, severity (sensory impairment may even further deteriorate the motor function), clinical course, treatment and reversibility. This should be evaluated overall but also within subgroups based on age and phenotype severity.

In addition, the feasibility of using SMA registries data to conduct a study is required as a Category 3 study in the RMP:

SMA Registries Study Feasibility Assessment (applicable for Itvisma only):

The overall objective of this feasibility assessment is to evaluate, in a structured manner, whether data from existing SMA registries are fit for purpose to evaluate the following in patients administered Itvisma:

- The incidence, risk factors, clinical course, and outcome of peripheral sensory neuropathy.
- Long-term patient follow-up (up to 15 years) with focus on the risk of tumorigenicity due to chromosomal integration.
- If effectiveness measures are routinely collected and available in the population not studied in the clinical studies. Of note, among patients with SMA Type 3 or 4, the maximum motor function is typically walking independently; thus, the effectiveness of treatment would be stabilization of maintenance of walking.

Milestones:

- Submission of a feasibility outcome report: within 3 months following the marketing authorization approval.
- Submission of a registry-based study protocol or alternative proposal as post-authorization measure: To be confirmed following the EMA assessment of the feasibility assessment results.

6.3. Plans for post-authorisation efficacy studies

There are no efficacy studies which are conditions of the marketing authorization for Itvisma.

6.4. Risk minimisation measures

6.4.1. Proposed risk minimisation measures

Routine risk minimisation measures

Description of routine risk minimization measures by safety concern (applicable for Zolgensma and Itvisma) proposed by the Applicant.

Table 38: Routine risk minimisation measures

Safety concern	**Routine risk minimization activities**
Hepatotoxicity (applicable for Zolgensma and Itvisma)	**Routine risk communication:** Zolgensma SmPC Sections 4.2, 4.4, 4.8, 5.2, 5.3 Zolgensma PL Sections 2, 3, 4 Itvisma SmPC Sections 4.2, 4.4, 4.8, 5.3 Itvisma PL Sections 2, 3, 4 **Routine risk minimization activities recommending specific clinical measures to address the risk:** ● Recommendations for monitoring of liver function tests prior to treatment and treatment with prednisolone in SmPC Sections 4.2, 4.4; PL Sections 2, 3 • Recommendations for routine monitoring of liver function tests in SmPC Section 4.4; PL Section 2 **Other routine risk minimization measures beyond the Product Information :** None
Transient thrombocytopenia (applicable for Zolgensma and Itvisma)	**Routine risk communication:** SmPC Sections 4.2, 4.4 and 4.8 PL Sections 2, 4 **Routine risk minimization activities recommending specific clinical measures to address the risk:** Recommendation for monitoring of platelet counts in SmPC Section 4.4 How to detect signs of low platelet counts in the PL Section 2 **Other routine risk minimization measures beyond the Product Information :** None
Thrombotic microangiopathy (applicable for Zolgensma and Itvisma)	**Routine risk communication:** Zolgensma SmPC Sections 4.2, 4.4, 4.8 Zolgensma PL Sections 2, 4 Itvisma SmPC Sections 4.2, 4.4 Itvisma PL Sections 2, 4 **Routine risk minimization activities recommending specific clinical measures to address the risk:** Requirement for Baseline laboratory testing of creatinine and complete blood count is mentioned in SmPC Section 4.2. Section 4.4 of the SmPC provides recommendation on close monitoring of platelet counts, and on testing for hemolytic anemia and renal dysfunction. It also recommends the need for urgent medical care if the physician and caregivers observe signs and symptoms of TMA. **Other routine risk minimization measures beyond the Product Information :** None
Dorsal root ganglia toxicity/peripheral sensory neuropathy (applicable for Zolgensma and Itvisma)	**Routine risk communication:** Zolgensma SmPC Section 5.3 Zolgensma PL: None Itvisma SmPC Sections 4.4, 4.8, 5.3

	Itvisma PL Section 2 **Routine risk minimization activities recommending specific clinical measures to address the risk:** Zolgensma: None Itvisma: Section 4.4 of the SmPC provides recommendation on neurological evaluation and other testing and/or symptom management. It also recommends the need for patients and caregivers to be informed about the signs and symptoms of peripheral sensory neuropathy and be advised to notify their physician promptly if such symptoms occur. PL Section 2 provides information about the signs of peripheral sensory neuropathy and advises to notify their physician promptly if such signs occur. **Other routine risk minimization measures beyond the Product Information:** None
Tumorigenicity due to chromosomal integration (applicable for Zolgensma and Itvisma)	**Routine risk communication:** SmPC Section 4.4 PL Section 2 **Routine risk minimization activities recommending specific clinical measures to address the risk:** None **Other routine risk minimization measures beyond the Product Information :** None
Long term safety of onasemnogene abeparvovec therapy (applicable for Itvisma only)	**Routine risk communication:** None **Routine risk minimization activities recommending specific clinical measures to address the risk:** None **Other routine risk minimization measures beyond the Product Information:** None

Additional risk minimisation measures:

The following educational materials are applicable and will be made available separately for Zolgensma and Itvisma:

Educational material

Healthcare professional guide

Objectives:

To mitigate possible risks before the start of treatment, at the time of the infusion, and after infusion by providing healthcare professionals with a guide on the following safety areas of concern:

- Hepatotoxicity
- Transient thrombocytopenia
- Thrombotic microangiopathy
- Dorsal root ganglia toxicity/peripheral sensory neuropathy (applicable for Itvisma only)

Rationale for the additional risk minimization activity:

To create a guide to help prescribers prepare a local checklist or summary materials to help to mitigate risks.

Target audience:

Healthcare professionals who are expected to prescribe, dispense and administer Zolgensma/Itvisma.

Plans to evaluate effectiveness of the risk minimization measures and criteria for success.

The effectiveness of risk mitigation of hepatotoxicity, transient thrombocytopenia, thrombotic microangiopathy and dorsal root ganglia toxicity/peripheral sensory neuropathy (applicable for Itvisma only) will be assessed in the context of risk evaluation in the PSUR.

Educational material

Caregiver information guide (applicable for Zolgensma)

Patient/patient's caregiver information guide (applicable for Itvisma)

Objectives:

To mitigate possible risks post-infusion by providing patients/caregivers with an educational guide on the following safety areas of concern:

- Hepatotoxicity
- Transient thrombocytopenia (low platelet count)
- Thrombotic microangiopathy
- Dorsal root ganglia toxicity/peripheral sensory neuropathy (applicable for Itvisma only)
- Other important aspects related with patient management (e.g. need for concomitant treatment with corticosteroids; monitoring vomiting to ensure corticosteroid uptake; signs and symptoms of infection; importance of observing overall health status including hydration, nutrition, and prevention of infections).

Rationale for the additional risk minimization activity:

To increase understanding of caregivers on the known risks which can be mitigated with actions before and following onasemnogene abeparvovec one-time administration, and with prompt contact of healthcare services.

Target audience:

Caregivers of patients in whom onasemnogene abeparvovec treatment is planned or who have received onasemnogene abeparvovec.

Plans to evaluate effectiveness of the risk minimization measures and criteria for success.

The effectiveness of risk mitigation of hepatotoxicity, transient thrombocytopenia (low platelet count), thrombotic microangiopathy and dorsal root ganglia toxicity/peripheral sensory neuropathy (applicable for Itvisma only) will be assessed in the context of risk evaluation in the PSUR.

Details of proposed additional risk minimization activities for Itvisma (from SmPC Annex II and RMP Annex 6) are presented below.

Key messages of the additional risk minimization measures
Healthcare Professional Information Pack

SmPC

Healthcare professional guide

The healthcare professional guide shall contain the following key messages (NOTE: Tradename is updated for each product: Zolgensma or Itvisma):

- Before the start of treatment:
  - Evaluate the patient's vaccination schedule;
  - [For Zolgensma] Inform the caregiver(s) about the main risks with Zolgensma and their signs and symptoms, including TMA, hepatic failure and thrombocytopenia; about the need for regular blood sampling; about the importance of corticosteroid medicine; practical advice concerning bodily waste disposal; [For Itvisma] Inform the caregiver(s) about the main risks with Itvisma and their signs and symptoms, including hepatotoxicity, thrombocytopenia, TMA and peripheral sensory neuropathy ; about the need for regular blood sampling; about the importance of corticosteroids;

- Inform the caregiver(s) of the need for increased vigilance in the prevention, monitoring, and management of infection before and after Tradename administration;
- Patients should be tested for the presence of AAV9 antibodies.

- At the time of treatment:
  - Check if the overall health status of the patient is suitable for administration of Tradename (e.g. resolution of infections) or if a postponement is warranted;
  - Check that corticosteroid treatment was started before the administration of Tradename;
- After treatment:
  - The corticosteroid treatment should continue for at least 2 months and not be tapered until AST/ALT are less than 2 x ULN, and all other assessments, e.g. total bilirubin, return to normal range;
  - Close and regular monitoring (clinical and laboratory) of the individual patient course should be performed for at least 3 months;
  - Prompt assessment of patients with worsening liver function tests and/or signs or symptoms of acute illness;
  - [For Zolgensma] If patients do not respond adequately to corticosteroids, or if liver injury is suspected, HCP should consult a paediatric gastroenterologist or hepatologist; [For Itvisma] If patients do not respond adequately to corticosteroids, or if liver injury is suspected, HCP should consult a gastroenterologist or hepatologist;
  - [For Zolgensma] If TMA is suspected, a specialist should be consulted. [For Itvisma] If TMA is suspected, a haematologist and/or nephrologist should be consulted.
  - [For Itvisma] Patients and caregivers should be informed about the signs and symptoms of peripheral sensory neuropathy and be advised to notify their physician promptly if such symptoms occur. If peripheral sensory neuropathy is suspected, complete neurological evaluation and other testing and/or symptom management should be considered based on the patient's clinical presentation.

Patient Information Pack

Package Leaflet

Caregiver information guide (applicable for Zolgensma)

Patient/patient's caregiver information guide (applicable for Itvisma)

The patient/patient's caregiver information guide shall contain the following key messages (NOTE: Tradename is updated for each product: Zolgensma or Itvisma):

- What is SMA.
- What is Tradename and how it works.
- Understanding the risks of Tradename.
- Treatment with Tradename: important information before, on the day of treatment and after treatment, including when to seek medical attention.
- It is recommended that patients present an adequate overall health status (e.g. hydration and nutritional status, absence of infection) prior to Tradename treatment, otherwise treatment may need to be postponed.
- [For Zolgensma and Itvisma] Tradename may increase the risk of abnormal clotting of blood in small blood vessels (thrombotic microangiopathy). [For Zolgensma] Cases generally occurred within the first two weeks after onasemnogene abeparvovec infusion. [For Zolgensma and Itvisma] Thrombotic microangiopathy is serious and may lead to death. Tell your or your child's doctor immediately if you notice signs and symptoms such as bruising, seizures or decrease in urine output. [For Zolgensma] Your child will have a regular blood test to check any decrease of platelets, the cells responsible for clotting, for at least 3 months after treatment. [For Itvisma] You or your child will have a regular blood test to check any decrease of platelets, the cells responsible for clotting, at least weekly for the first month after treatment. [For Zolgensma and Itvisma] Depending on the values and other signs and symptoms, further evaluations may be required.
- [For Zolgensma and Itvisma] Tradename can lower blood-platelet counts (thrombocytopenia). [For Zolgensma] Cases generally occurred within the first three weeks after onasemnogene abeparvovec infusion. [For Itvisma] Cases generally occurred within the first week after the patient was given Itvisma. [For Zolgensma] Possible signs of a low blood-platelet count you need to look out for after your child is given Zolgensma include abnormal bruising or bleeding. Speak to your doctor if you see signs such as bruising or bleeding for longer than usual if your child has been hurt. [For Itvisma] Possible signs of a low blood-platelet count you need to look out for after you or your child is given

Itvisma include abnormal bruising or bleeding. Speak to your or your child's doctor if you see signs such as bruising or bleeding for longer than usual if you or your child has been hurt.

- [For Zolgensma and Itvisma] Tradename can lead to an increase in enzymes (proteins found within the body) produced by the liver. In some cases, Tradename can affect the function of the liver and lead to injury of the liver. [For Zolgensma] Injury to the liver can lead to serious outcomes, including liver failure and death. [For Zolgensma] Possible signs you need to look out for after your child is given this medicine include vomiting, jaundice (yellowing of the skin or of the whites of the eyes), or reduced alertness. Tell your child's doctor straightaway if you notice your child develops any symptoms suggestive of injury to the liver. Your child will have a blood test to check how well the liver is working before starting treatment with Zolgensma. Your child will also have regular blood tests for at least 3 months after treatment to monitor for increases in liver enzymes. Depending on the values and other signs and symptoms, further evaluations may be required. [For Itvisma] Possible signs you need to look out for after you or your child is given this medicine include vomiting, jaundice (yellowing of the skin or of the whites of the eyes), or reduced alertness. Tell your or your child's doctor straight away if you notice any symptoms suggestive of injury to the liver. You or your child will have a blood test to check how well the liver is working before starting treatment with Itvisma. You or your child will also have regular blood tests for at least 3 months after treatment to monitor for increases in liver enzymes. Depending on the values and other signs and symptoms, further evaluations may be required.
- [For Zolgensma] Your child will be given a corticosteroid medicine such as prednisolone before treatment with Zolgensma, and for about 2 months or longer after Zolgensma treatment. The corticosteroid medicine will help manage effects of Zolgensma such as increase in liver enzymes that your child could develop after treatment with Zolgensma. [For Itvisma] You or your child will be given a corticosteroid medicine such as prednisolone before treatment with Itvisma, and for about 2 months or longer after Itvisma treatment. The corticosteroid medicine will help manage effects of Itvisma such as increase in liver enzymes that you or your child could develop after treatment with Itvisma.
- [For Zolgensma] Tell your doctor in the event of vomiting before or after treatment with Zolgensma, to make sure that your child does not miss corticosteroid dosing. [For Itvisma] Tell your or your child's doctor in the event of vomiting before or after treatment with Itvisma, to make sure that you or your child does not miss corticosteroid dosing
- Before and after treatment with Tradename it is important to prevent infections by avoiding situations that may increase the risk of the patient getting infections. Caregivers and close contacts with the patient should follow infection prevention practices (e.g. hand hygiene, coughing/sneezing etiquette, limiting potential contacts). Inform the doctor straight away in case of signs and symptoms suggestive of infection, such as respiratory infection (coughing, wheezing, sneezing, runny nose, sore throat or fever) prior to administration as the administration may need to be delayed until the infection is resolved, or after treatment with Tradename as it may lead to medical complications that may require urgent medical attention.
- [For Itvisma] Tell your or your child's doctor immediately if you or your child experience numbness, tingling, prickling or pain in the arms, hands, legs and/or feet after being given Itvisma. These may be signs of peripheral sensory neuropathy.
- Useful further information (supportive care, local associations).
- Contacts of the physician/prescriber.

6.4.2. Discussion on the risk minimisation measures

6.4.2.1. Routine risk minimisation measures

The proposed routine risk minimisation measures are agreed. However, additional risk minimisation measures are considered key for a positive benefit-risk balance.

6.4.2.2. Additional risk minimisation measures

Educational material

Educational materials are important for prescribers and patients, especially since this is a one-time treatment where any shortcomings in information and preparation may be impossible to correct

afterwards. The need for and the key messages of the educational material are included as an Annex II condition.

6.5. RMP summary and RMP annexes overall conclusion

The RMP Summary and the annexes are considered acceptable.

6.6. Overall conclusion on the Risk Management Plan

The CHMP considers that the risk management plan version 5.3 is acceptable.

7. Pharmacovigilance

7.1. Pharmacovigilance system

The CHMP considers that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

7.2. Periodic safety update reports (PSURs) submission requirements

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.

8. Product information

8.1. User consultation

8.1.1. Conclusion from the checklist for the review of 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.

8.2. 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 for the following reasons: This product will be used in hospital setting only.

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 the EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD group.

8.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Itvisma is included in the additional monitoring list since it is a biological medicine.

Therefore, the summary of product characteristics and the package leaflet include a statement that this medicinal product is subject to additional monitoring and will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

9. Benefit-risk assessment

9.1. Therapeutic context

9.1.1. Disease or condition

For a detailed description, please refer to section 3.1 of this document.

Spinal muscular atrophy (SMA) is a rare (1:10.000) autosomal-recessive neuromuscular disease caused by bi-allelic loss of the *SMN1* gene at locus 5q13, which encodes the Survival Motor Neuron (SMN) protein. SMN protein depletion causes degeneration of motor neurons in the brainstem and spinal cord, with consequent progressive muscular atrophy. Disease severity is, for the most part, inversely correlated with the copy number (1 to 5) of the *SMN2* gene, a partially functional copy of *SMN1,* each producing a proportion of the SMN protein.

Four clinical subtypes (Type 1 to 4) have been identified in SMA based on age of onset and motor milestones achieved without treatment, which together constitute a spectrum of conditions ranging from severe forms with neonatal onset, to milder forms manifesting in later life. SMA Type 1 is the most common (45-60%) and severe phenotype, manifesting before 6 months of age, where children never gain the ability to sit and typically do not survive after 2 years of age without treatment. SMA Type 2 (20-30%) has onset within 18 months of age, children are able to sit unassisted but never walk independently. SMA Type 3 patients (~10-20%) attain independent walking, then may lose this ability [type 3a has onset between 18-36 months; type 3b has onset >3 years]. SMA Type 4 (<5%) is an adult onset disease with mild symptoms.

With the advent of disease-modifying therapies, and the changed clinical picture of treated patients, a functional classification based on the maximum motor function achieved ('Non-sitters', 'Sitters', 'Walkers') can be used.

The clinical diagnosis of SMA is confirmed by genetic testing. Several countries in Europe have implemented SMA in their newborn screening programmes.

9.1.2. Available therapies and unmet medical need, proposed therapeutic indication

Disease-modifying therapies currently approved in Europe for SMA are: onasemnogene abeparvovec administered IV (Zolgensma), and two splicing modulators of the *SMN2* gene, i.e., nusinersen (Spinraza) and risdiplam (Evrysdi).

Zolgensma (onasemnogene abeparvovec) has been approved by the CHMP in 2020, for the treatment of patients with 5q 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 *SMN2* gene.

Onasemnogene abeparvovec is an adeno-associated virus serotype 9 (AAV9) vector gene therapy containing a functional copy of *SMN1*. Zolgensma is administered as a one-time IV injection, at the dose of 1.1 x 10^{14} per kg up to 21 kg bodyweight. Efficacy and safety data in patients ≥2 years or >13.5 kg bodyweight is limited.

Spinraza (nusinersen) has been approved by the CHMP in 2017, for the treatment of 5q SMA. Nusinersen is an antisense oligonucleotide that blocks a splice silencer within intron 7 of *SMN2* pre-mRNA, thus increasing the production of full-length mRNA and SMN protein. Nusinersen is administered intrathecally (IT), and requires lifelong injections every 4 months, which is not feasible for every patient, and associated with safety risks.

Evrysdi (risdiplam) has been approved by the CHMP in 2021, for the treatment of 5q SMA in patients with a clinical diagnosis of SMA Type 1, Type 2 or Type 3, or with one to four *SMN2* copies. Risdiplam is a small molecule that facilitates *SMN2* exon 7 retention within the mRNA and thus increases SMN protein levels. Risdiplam is administered orally once a day for life.

In sum, multiple treatment options are available for patients with SMA, including one for all SMA types (nusinersen). However, the possibility of using these treatments can be limited, due to burden of life-long administrations (nusinersen and risdiplam), and indication/posology restrictions (Zolgensma and risdiplam).

The proposed product Itvisma (also referred to as OAV101B or Itvisma in this report), contains the same active substance as Zolgensma, but is designed for single-dose IT administration, instead of IV as for Zolgensma. Rationale of the proposed IT formulation is delivery of onasemnogene abeparvovec directly into the CSF of the spinal cord, using a single fixed dose (1.2x10^{14} vg) irrespective of the patient's bodyweight. This would permit to reduce total vector load, and to treat older and heavier SMA patients. The single administration would represent a therapeutic advantage, as other treatment options for these patients (nusinersen and risdiplam) require lifelong administrations.

The final indication for OAV101B (Itvisma) is:

"*… for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene in patients 2 years of age and older*."

9.2. Main clinical studies

Pivotal study

Study **B12301** was a 52-week phase III, randomized (3:2), double-blind, sham-controlled study, that enrolled 126 treatment-naïve SMA patients (OAV101B =75, Sham=51), ≥2 to <18 years of age, a clinical onset (as reported by caregivers) between 6 and 33 months, and able to sit but never able to walk independently. This translates to SMA type 2. Genetically, patients had mostly 3 copies of the *SMN2* gene (n=119/94%), with the remaining patients having either 2 or ≥4 *SMN2* copies.

Primary endpoint was change-from-baseline in the HFMSE (Hammersmith Functional Motor Scale – Expanded) total score at week 52 in the overall study population (2 to <18 years). Key secondary (or co-primary) endpoint was the same score at week 52 in the 2 to <5 years age group.

Another key secondary endpoint was change-from-baseline in the RULM (Revised Upper Limb Module) score at week 52 in the overall study population.

A change in the HFMSE score of at least 3 points is considered clinically meaningful. Accordingly, additional secondary endpoint was the proportion (%) of patients with a HFMSE ≥3-point improvement from baseline to week 52 in the overall study population.

Supportive study

Data for patients aged 6 to <24 months derive from study **CL-102**.

Study CL-102 was a phase I/II open-label, dose ranging study that in cohort 2 (therapeutic dose) enrolled 25 treatment-naïve SMA patients aged 6 to <60 months. Patients had 3 copies of *SMN2*, clinical onset at <12 months of age, and were able to sit but not to stand or walk independently. This translates to SMA type 2.

Patients were stratified into two age groups at dosing, with specific study endpoints:

- 6 to <24 months (n=13), primary endpoint: proportion of patients who achieved to stand alone;
- 24 to <60 months (n=12), primary endpoint: change from baseline in HFMSE.

Safety data from Study B12301 sham-controlled 52-week Period 1 form the basis for the primary safety assessment. Safety data from participants 6 months or older who received OAV101B at the $1.2x10^{14}$ vg dose and were followed until week 52 in studies B12301 period 1, B12302 (n= 27) and CL-102 (n= 25) were pooled in the '52-week pooled analysis'. In the 'long-term safety analysis', safety data was pooled from participants 6 months or older who received OAV101B at the $1.2x10^{14}$ vg dose from ongoing study B12301 Period 2 (n= 40), and open-label extension studies A12308 and LT-002.

9.3. Favourable effects

The primary endpoint of the pivotal study (B12301) was met. The least square mean (LSM) change from baseline in HFMSE total score in overall population was 2.39 (95% CI: 1.52 ; 3.26) for OAV101B, and 0.51 (95% CI: -0.55 ; 1.56) for Sham, with a treatment difference of 1.88 (95% CI: 0.51 ; 3.25; p-value=0.0074).

This finding was supported by the proportion of patients who achieved a clinically meaningful improvement in HFMSE (≥3 points), which was 39.2% (95% CI: 28.07 ; 50.31) in the OAV101B group, and 26.0% (95% CI: 13.84 ; 38.16) in the Sham group, although the difference did not meet statistical significance with an odds ratio (OR) of 2.03 (95% CI: 0.90 to 4.57; p-value=0.0879).

Improvement in the primary outcome was supported by a numerical improvement in the key secondary outcome: the LSM change from baseline in RULM total Score in the overall population was 2.44 (95% CI: 1.69 ; 3.19) for OAV101B vs 0.92 (95% CI: 0.00 ; 1.83) for Sham, with a LSM difference of 1.52 (95% CI: 0.34 ; 2.71; p-value=0.0122).

Supportive evidence of efficacy derives from study CL-102 in the 2 to <5 years age group: the LSM change from baseline at 12 months in HFMSE was 6.0 (95% CI: 3.7- 8.3).

9.3.1. Uncertainties and limitations about favourable effects

SMA is a wide and heterogenous spectrum of conditions in terms of severity, which can be classified into 1-4 subtype based on age at symptom onset and motor milestone achievement.

SMA type data was not collected in the pivotal study. The study population comprised treatment naïve sitting and never-ambulatory patients with symptom onset after the age of 6 months, hence is considered restricted to patients with SMA type 2. Treatment naïve patients with the most severe phenotype and onset <6 months (type 1), and late onset ambulatory patients with milder phenotypes (type 3: onset >18 months, and type 4: adult onset) were not included in the development program. While extrapolation of efficacy from SMA type 2 to type 3 and 4 (milder forms) has been discussed adequately and is considered justified, this is not the case for the most severe form, i.e. type 1. The

target population of Itvisma therefore excludes treatment-naïve SMA type 1.

The pivotal study population was aged ≥2 to <18 years. Data for the 6 months-2 years age group derived from a small sample of a supportive open label study (CL-102). In this study efficacy was not demonstrated: the primary endpoint was not met, and the secondary endpoints were not supportive of efficacy in this age group. In addition, the adequacy of the fixed dose, proportionally higher in 6 months-2 years children, was not supported by the provided non-clinical NPH studies. Given the limitations of study CL-102 and the lack of demonstrated efficacy, additional discussion on extrapolation of efficacy (and safety) from children aged ≥2 to <18 years to children aged 6 months-2 years was required. Based on the provided argumentations, a clearer absolute improvement was expected in these younger patients, similarly to the younger age-group in the pivotal study. The proposed target population from 6 months of age is therefore also not convincingly supported by the extrapolation exercise. Therefore, patients aged 6 months to 2 years have been excluded from the indication. The exclusion of this age-group also excludes patients treatment-naïve type 1, due to their natural disease course.

Pivotal study participants were reported of never being able to walk independently, which does not fit the reported age of symptom onset up to 33 months (a delay in acquiring ambulation is a key symptom of SMA). Age at onset data collection solely relied on caregivers' reports, which yielded inaccurate data and clinically implausible 'age at onset and highest motor function achieved' combinations. Consequently, the provided age at onset data are considered unreliable, as well as any derived measure (like disease duration) or analysis based on this data. This issue is not considered solvable, methodologically. Therefore, the patients' baseline clinical characterization solely relies on highest motor function achieved and motor function at the start of treatment (baseline HFMSE).

Subgroup analyses showed somewhat unexpected results, with older children (5 to <18 years) demonstrating greater and more consistent motor gains than younger children (2 to <5 years) compared to sham, possibly reflecting differences in disease progression across age and development.

The absence of adult participants in the trial required extrapolation of efficacy data to older SMA patients, where disease progression and motor neuron reserve may differ significantly. Although the mechanism of action of OAV101B suggests biological plausibility for benefit across ages, this may differ per SMA type. The inclusion of adult patients is supported by the provided extrapolation exercise.

SMA is a lifelong disease. Effectiveness of OAV101B was evaluated at 52 weeks in study B12301. Thereafter, long term efficacy was assessed in two long term follow-up studies where concomitant disease-modifying therapies were permitted. No conclusions can be made on long term efficacy of OAV101B in isolation. On the other hand, the use of add-on treatments is considered unavoidable, especially in the long-term, and in the current scenario where other therapeutic options, with different (and complementary) mechanism of action, are available.

9.4. Unfavourable effects

In study B12301 Period 1, 97.3% of participants in OAV101B group and 90.2% of participants in the sham group experienced at least one adverse event (AE). Serious adverse events (SAEs) were reported in 28.0% and 33.3% of the OAV101B and sham group respectively and 1 participant in each treatment arm discontinued the study due to an AE. The most common treatment-emergent adverse events (TEAEs) (OAV101B vs. sham) were: *upper respiratory tract infection* (34.7% vs. 29.4%), *pyrexia* (25.3% vs. 23.5%) and *vomiting* (20.0% vs. 11.8%).

In the 52-week pooled safety analysis, which includes 127 participants treated with OAV101B, 98.4% experienced any AE and 24.4% reported SAEs. One participant discontinued one of the studies in the pool due to an AE. The most common TEAEs reported in this pool were *pyrexia* (36.2%), *upper*

respiratory tract infection (34.6%) and *vomiting* (28.3%).

Events related to thrombocytopenia were reported in 5.3% of the OAV101B and 3.9% of the sham treated participants. The events were mild and transient. Transient increases in hepatic enzymes (ALT or AST) were observed following OAV101B treatment. None of the participants in the pivotal study and the 52-week pooled analysis had increases in total bilirubin > 1.5x ULN and none met the criteria for Hy's Law.

Dorsal root ganglia (DRG) toxicity was previously identified in non-clinical studies, which presents itself as peripheral sensory neuropathy in humans. In the overall OAV101B clinical development plan there were 4 (3.1%) cases of peripheral sensory neuropathy pertaining to events of hypoesthesia and paraesthesia. Three cases (2 in study 12301 Period 1 and 1 in study B12302) were considered related to OAV101B treatment of which 2 had persistent decreased sensory nerve action potential (SNAP) amplitudes and symptoms requiring treatment as of the recent data cut-off in the follow-up. One was reported in the sham group.

The safety profile of OAV101B is quite similar to what has been previously been observed with onasemnogene abeparvovec intravenous administration (Zolgensma, EMEA/H/C/004750). Thus there is a large overlap in identified ADRs of OAV101B and Zolgensma, although specific events (e.g. thrombocytopenia, hepatic enzymes increased) are observed with lower frequency which may be related to less systemic exposure due to the intrathecal route of administration.

9.4.1. Uncertainties and limitations about unfavourable effects

The risk for DRG toxicity/peripheral sensory neuropathy following OAV101B cannot be excluded. In 2/3 cases attributed to OAV101B treatment, persistent reductions in SNAP amplitudes were seen the lower extremities of two participants, of which in one progression to the upper extremities was observed. These two cases are persistent, i.e., the AESI has not been resolved in both cases. Both participants continue to have symptoms despite ongoing treatment in the long term extension studies. Additional information has been provided with respect to OAV101B efficacy in these participants, but the impact of peripheral sensory neuropathy on OAV101B efficacy cannot be determined. A dedicated PASS is needed to further caracterise this adverse reaction.

Additional discussion has been provided on (early) signs and symptoms, treatment and early identification strategies. With respect to the two persistent cases there does not appear to be a pattern or a specific sequence of events. Based on the totality of information available on the two persistent cases of neuropathy it is agreed with the Applicant that there are no risk factors or any additional identification strategies that could be included in the SmPC. There is no clear pattern in terms of signs and symptoms that would be informative for HCP's and caregivers to pay particular attention to. SNAP measurement does not appear to be predictive for the sensory neuropathy, given that there have been patients with symptoms and normal SNAP and vice versa. Based on the available data the warning as currently incorporated in section 4.4 of the SmPC is sufficient.

There were no patients with anti-AAV9 antibody titres > 1:50 and higher vector loads required included in the study. The impact on the efficacy and safety in this population is unknown, therefore an appropriate warning is included in the SmPC. Though tumorigenicity due to integration of the vector DNA into the genome has been reported for another investigational product, the risk is considered potential. This risk can be further monitored in the regular PSURs and no additional monitoring or studies are required.

The current follow up of 2-3years is insufficient to meet the requirements of long-term follow-up for an ATMP product. Therefore a dedicated disease registry-based category 3 PASS with systematic data

collection is required.

For patients aged 6 months - < 2 years the available data derives from a small sample of a supportive open label study. The available safety data suggests no major differences between the younger and older age cohorts. However, the available data is limited, only 13 patients were treated with the target dose of OAV101B. Furthermore, it is noted that the proposed fixed dose of OAV101B could result in a higher exposure in the youngest age group (1.6-fold higher). Comparative analyses between dose levels evaluated in study CL-102 suggest no major differences in safety profile. However, the subgroups are small which complicates definitive conclusions on the higher exposure. Considering the uncertainty regarding the available safety data in in patiens aged 6months to<2 years of age, this population is currently excluded from the indication.

9.5. Effects table

Table 39: Effects Table for Itvisma, indication: "…for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene in patients 2 years of age and older."

Effect (short description)	Treatment	Control	Uncertainties/ Strength of evidence	Ref
Favourable effects				
	OAV101B 1.2x10^{14} vg	**Sham**		
HFMSE change from baseline in overall population at week 52 LSM (95% CI)	2.39 (1.52 - 3.26)	0.51 (-0.55 - 1.56)	**SoE:** difference 1.88 (0.51 - 3.25); p-value=0.0074).	Study B12301
RULM change from baseline in overall population at week 52 LSM (95% CI)	2.44 (1.69 - 3.19)	0.92 (0.00 - 1.83)	**SoE**: Difference: 1.52 (0.34 - 2.71); p-value=0.0122	Study B12301
Unfavourable effects				
Signs and symptoms suggestive of DRG toxicity - including peripheral sensory neuropathy N / total (%)	2/75 (2.7)	1/51 (2.0)	**SoE:** Low frequency of cases observed across the clinical studies 1 case in sham treatment which was transient (1 day) **Unc:** 2 cases in OAV101B were persistent and ongoing at last follow-up. (early) Identification, progression, treatment and reversibility of events remain unclear	Study B12301
Upper respiratory tract infection N / total (%)	26 / 75 (34.7)	15/51 (29.4)	**SoE:** - **Unc:** Similar frequencies observed in treatment / sham group	Study B12301

Abbreviations: Ref: reference; Unc: uncertainties; SoE: strength of evidence; HFMSE: Hammersmith Functional Motor Scale Expanded; RULM: Revised Upper Limb Module; LSM: least square mean; CI: Confidence interval ; PE: primary endpoint; SE: secondary endpoint; OR: odds ratio.

9.6. Benefit-risk assessment and discussion

9.6.1. Importance of favourable and unfavourable effects

Clinical efficacy

SMA is a progressive neurodegenerative disease, and all patients lose function over time. Efficacy of OAV101B was demonstrated in patients with type 2 SMA aged 2 to 18 years by significant improvement after one year in two validated and complementary functional outcome measures of SMA, i.e. HFMSE, assessing daily living activities, and RULM, assessing functionality of the upper limb which is critical in non-ambulatory patients. Inconsistent results between age groups were explained by different pattern of disease progression across age and development, hence efficacy is considered demonstrated across the entire age range investigated.

Patients with type 1, type 3 and type 4 were not included in the development program.

Extrapolation of efficacy to type 1 is not possible, since no data were provided in these clinically more severe, rapidly progressing, and more vulnerable patients than those investigated in the development program. The timing of the administration would not be optimal for type 1, since clinical onset is before the applied 6 months of age, and early treatment is critical for increasing the therapeutic effect (Strauss et al., Nat Med. 2022). The suitability of the IT formulation per se is also questioned in type 1, given visceral involvement, and the reduced systemic distribution of OAV101B. The efficacy (and safety) of OAV101B is not established in this more severe and vulnerable patient group, which therefore have been excluded from the indication.

Conversely, extrapolation to patients with milder/later onset SMA forms than those included in the development program (i.e. type 3 and 4), as well as in adult patients, is considered sufficiently justified by the provided subgroup analyses in patients groups with different functional levels. The severity of the SMA phenotype correlates with the age of symptoms onset. Due to unreliable age at onset data, considerations based on age at onset and treatment effect, which would have helped informing extrapolation to later onset SMA forms, cannot be made. This uncertainty however does not hamper extrapolation: there is sufficient evidence from the data that the product could be efficacious in later onset forms, and no indication (from disease pathogenesis, mechanism of action, and experience with other SMN-targeted therapy) suggesting otherwise.

Pivotal evidence derived from patients 2-18 years of age. Supportive data in patients aged 6 months-2 years derived from a small open label study and are overall inconclusive for efficacy, whereas a clear (and even higher) effect was expected in this age group based on the observed age effect in the pivotal population (and therefore, the provided extrapolation exercise from the older age-group is also considered unsuccessful). The proportionally higher dose (up to 1.6 folds) in these younger patients has not been substantiated by the provided non-clinical data. Hence, there are concerns pertaining dose and efficacy in patients 6 months-2 years of age, which have been reflected in the SmPC.

Therefore, the proposed broad indication in all SMA types from the age of 6 months was not supported, as it should exclude patients with SMA type 1, and patients aged 6 months-2 years in general (irrespective of their SMA type). By restricting the indication from the age of 2 years, both issues have been solved due to the natural disease course of SMA type 1. The window of opportunity for treatment-naïve type 1 patients would have passed by that age anyway.

SMA is a genetic chronic disease and OAV101B administration cannot be repeated, hence maintenance of efficacy is essential. Long-term efficacy was evaluated in two open label supportive studies where

concomitant disease-modifying therapies were used, hence no conclusions can be drawn on maintenance of efficacy of OAV101B in isolation. This is of relevance for all SMA types, and complicates extrapolation to late onset forms at the mild end of the spectrum (type 3 and 4), for which no data were provided. In light of the notion that early treatment is key in SMA –as other neurodegenerative diseases-, and that long term efficacy of OAV101B is unknown, it is difficult to estimate *when* the product should be administered in later onset forms. However, these uncertainties are not considered likely to negatively impact the overall benefit-risk of the product. Long-term efficacy and residual uncertainties pertaining late-onset forms can be addressed post-marketing.

The reported critical GCP findings likely did not affect the reliability of relevant efficacy and safety data for the assessment of the B/R.

Clinical safety

The safety profile of OAV101B is quite similar to what has previously been observed with onasemnogene abeparvovec intravenous administration (Zolgensma, EMEA/H/C/004750). Thus there is a large overlap in identified ADRs of OAV101B and Zolgensma, although specific events (e.g. thrombocytopenia, hepatic enzymes increased) are observed with lower frequency which may be related to less systemic exposure due to the intrathecal route of administration.

DRG toxicity was previously identified in non-clinical studies and three cases of peripheral sensory neuropathy associated with OAV101B treatment have been reported in the clinical development plan. Given the persistent nature of the two cases from study B12301 Period 1, the risk for peripheral sensory neuropathy following OAV101B cannot be excluded. The impact on OAV101B efficacy cannot be determined based on these two cases. Since limited information is available, a pattern of events, risk factors, early identification strategy and reversibility/treatment remain uncertain. The available safety data suggest no major differences between the younger (6 months – 2 years) and older age cohorts. However, the available data is limited, only 13 patients were treated with the target dose of OAV101B. Considering the uncertainty whether the safety data base for this population is fully captured for OAV101B currently a restriction of the indication to exclude children <2 years of age is a necessity.

9.6.2. Balance of benefits and risks

Efficacy has been shown on different functional measures of SMA in treatment naïve patients with SMA type 2 across the entire 2 to <18 years age range. Extrapolation of the indication to patients with milder SMA forms than those included in the development program (type 3 and 4), as well as in adult patients, is considered justified from the efficacy and safety perspective. Therefore, the therapeutic indication of Itvisma can include all SMA patients across the spectrum from the age of 2 years, without further specification.

Based on the totality of information available on peripheral sensory neuropathy, there are no risk factors, additional identification strategies, nor clear pattern in terms of signs and symptoms that would be informative for HCPs and caregivers to pay particular attention to. The currently available information is adequately reflected in the SmPC.

The submitted data support a favourable benefit risk balance in the following indication:

"… for the treatment of 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the SMN1 gene in patients 2 years of age and older."

9.6.3. Additional considerations on the benefit-risk balance

9.6.3.1. Third party intervention(s)

The following input was received from the patient organization SMA Europe (summarized):

"The intrathecal formulation of onasemnogene abeparvovec has the potential to extend the option of a one-time treatment approach to a broader SMA population, which we welcome. Therefore, at the outset, we wish to clearly articulate two overarching considerations.

First, we support broad and equitable access to OAV101 IT through a non-restrictive indication, provided that its safety and efficacy are established.

Second, we strongly support systematic post-authorisation evidence generation to address residual uncertainties, in particular regarding long-term safety, long-term effectiveness, and outcomes of relevance to people living with SMA. We view these considerations as complementary and essential to informed clinical decision-making over time."

CHMP position:

The SMA Europe patient advocate input is valuable. Main points of considerations are 1) support of a broad non-restrictive indication, provided that its safety and efficacy are established, and 2) support of systematic post-authorisation evidence generation to address residual uncertainties.

Both points of considerations have been taken into account in the current assessment procedure.

1. Upon evaluation of the provided clinical efficacy and safety data, a favorable B/R profile has been estimated for treatment naïve patients with SMA type 2, type 3 and type 4, and for treatment experienced patients from the age of 2 years. Consequently, the therapeutic indication can indeed be non-restrictive by including all SMA patients **across the spectrum from the age of 2 years**, with no further specification -phenotype or genotype-.

   The provided safety and efficacy data were considered *not sufficient* to estimate a favorable B/R profile for patients below the age of 2 years or for treatment naïve SMA type 1, hence these patients must be excluded from the indication. This won't represent a problem for these patients, as can be treated with Zolgensma, and there is no comparative data nor indication of a therapeutic advantage of Itvisma compared to Zolgensma from either the efficacy or the safety perspective.

2. Despite the B/R is estimated to be favorable in the broad ≥2 years SMA patient population, residual uncertainties pertaining long-term efficacy, long-term safety, and the expected benefit / therapeutic window for patients not included in the development program (ambulant, adults) remain. Notably however, these uncertainties are not considered likely to influence the B/R profile of the product. There are two ongoing long-term follow-up studies, and in addition the Applicant has been required to propose a post-authorisation safety study (PASS). Therefore, the patient representative recommendation of generating **post-authorisation evidence** will be addressed by these studies, for both the safety and efficacy perspective.

9.7. Benefit-risk conclusions

9.7.1. Final CHMP conclusions

The overall benefit/risk balance of Itvisma is **positive**.