22 February 2024
EMA/276404/2024
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Qalsody

International non-proprietary name: tofersen

Procedure No. EMEA/H/C/005493/0000

Note

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

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List of abbreviations

aPPT	activated partial thromboplastin time
A5V	p.Ala5Val
ABL	Pooled group from Study 101 Part A and B and Study 102
ADA	Antidrug antibody
ADR	Adverse drug reaction
(S)AE	(Serious) Adverse event
AESI	Adverse events of special interest
ALT	Alanine transaminase
ALS	Amyotrophic lateral sclerosis
ALSAQ-5	ALS Assessment Questionnaire
ALSFRS-R	ALS Functional Rating Scale-Revised
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ASO	Antisense oligonucleotide
AST	Aspartate transaminase
$AUC_{(0\text{-}24/48/last)}$	Area under the concentration time curve (from time 0 to 24/48 hours / to the last measured concentration)
BSA	Body surface area
BUN	Blood urea nitrogen
CC	Correlation coefficients
CDF	Cumulative Distribution Function
CDP	Clinical development program
CHL	Chinese Hamster Lung
CI	Confidence interval
CIR	Copy-increment-from-reference
CL	Clearance
CL	Pooled group from Study 101 Part C and Study 102
CL1	Early start tofersen treatment group (pooled group from Study 101 Part C tofersen and study 102)
CL2	Placebo-delayed start tofersen treatment group (pooled group from Study Part C placebo and Study 102)
CMA	Conditional marketing authorisation
CMAP	Compound muscle action potential
C_{max}	Maximum observed concentration
CNS	Central nervous system
CR	Copy-reference
CSA	Country-specific amendment
CSF	Cerebrospinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
CYP450	Cytochrome P450

DART	Developmental and reproductive toxicity
DDI	Drug-drug interaction
DNA	Deoxyribonucleic acid
EAP	Expanded access program
EC_{50}	half maximal effective concentration
ECG	electrocardiogram
ED_{50}	Half-maximal effective dose
EFD	Embryo-foetal development
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EOS	End of study
EQ-5D-5L	EuroQol 5 Dimension, 5 Level Questionnaire
ERA	Environmental risk assessment
ES	Early start
fALS	familial ALS
FDA	United States Food and Drug Administration
FEFD	Fertility and embryo-foetal development
FPS	Faster progressing group
FSS	Fatigue Severity Scale
GD	Gestation day
GGT	Gamma-glutamyl transferase
HCP	Health care professional
HED	Human equivalent dose
hERG	human ether-à-go-go related gene
HHD	Hand held dynamometry
HR	Hazard ratio
K_A	Absorption rate
IC_{50}	Half-maximal inhibitory concentration
ICV	Intracerebroventricular
IDMC	Independent Data Monitoring Committee
IIV	Inter-individual variability
IT	intrathecal
J2R	Jump to reference
JRT	Joint rank test
K	Elimination rate
LD	Lactation day
LFT	Liver function test
LS	least squares
LTE	Long-term extension
MAD	Multiple ascending dose
MAR	Missing at random

MAUEC	Marketing Authorisation under Exceptional Circumstances
M(C)ID	Minimally (clinically) important difference
MI	multiple imputation
(m)ITT	(modified) intent-to-treat
MRI	Magnetic Resonance Imaging
mRNA	messenger ribonucleic acid
N/A	Not applicable
NCA	Non-compartmental analysis
NE	Not estimable
NfH	Neurofilament heavy chain
NfL	Neurofilament light chain
NHP	Nonhuman primate
NOAEL	No observed adverse effect level
non-mITT	Non-modified intent-to-treat
NR	Not reported
OLE	Open-label extension
PBT	Persistence bioaccumulation toxicity
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
pNfH	Phosphorylated neurofilament heavy chain
popPK	Population PK
PRO	Patient-reported outcome
PT	Preferred term
PV	permanent ventilation
SAD	single-ascending dose
sALS	sporadic ALS
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation
SE	Standard error
SMQ	Standardized Medical Dictionary for Regulatory Activities Query
SOB	Specific Obligation
SOC	System organ class
SOD1	Superoxide dismutase-1
SOD1-ALS	ALS associated with a mutation in the *SOD1* gene
SPS	Slow progressing group
Study 101 Part A	Study 233AS101 Part A SAD
Study 101 Part B	Study 233AS101 Part B MAD
Study 101 Part C	Study 233AS101 Part C Phase 3
Study 102	Study 233AS102 LTE
SVC	Slow vital capacity

$t_{1/2}$	Elimination half-life
TEAE	Treatment-emergent adverse event
t_{max}	Time of maximal concentration
TK	Toxicokinetic
$V_{central}$ V_2	Central volume of distribution
$V_{peripheral}$	Peripheral volume of distribution
ULN	Upper Limit of Normal

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Biogen Netherlands B.V. submitted on 31 October 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Qalsody, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 14 November 2019.

Qalsody was designated as an orphan medicinal product EU/3/16/1732 on 29 August 2016 in the following condition: treatment of amyotrophic lateral sclerosis.

The applicant applied for the following indication: the treatment of adults with amyotrophic lateral sclerosis (ALS), associated with a mutation in the superoxide dismutase 1 (SOD1) gene.

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

1.2. Legal basis, dossier content

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, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0337/2018 on the granting of a product-specific waiver.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. Marketing authorisation under exceptional circumstances

The applicant requested consideration of its application for a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation.

1.5.2. New active Substance status

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

1.6. Protocol assistance

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

Date	Reference	SAWP co-ordinators
26 April 2019	EMEA/H/SA/4090/1/2019/PA/III	Dr. Marion Haberkamp Dr. Armando Magrelli
25 June 2020	EMEA/H/SA/4090/2/2020/PA/I	Dr. Marion Haberkamp Dr. Finbarr Patrick Leacy

The protocol assistance pertained to the following quality, non-clinical, and clinical aspects.

- Sufficiency of the manufacturing process, analytical and facility for registration of the ready-to-fill drug substance; whether the drug substance process development and validation programs have demonstrated the ability to consistently deliver ready-to-fill drug substance; the proposed adjustments to presentation of the data reflecting the facility, process and control strategy for tofersen manufacture in a modified CTD content structure.
- Adequacy of the proposed non-clinical data package for MAA.
- The design of the planned pivotal study and, in particular, the population, primary and secondary endpoints, dose selection, study duration, sample size and statistical analysis; the proposed clinical data package for MAA.

1.7. Steps taken for the assessment of the product

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

Rapporteur: Martina Weise Co-Rapporteur: Alexandre Moreau

The application was received by the EMA on	31 October 2022
The procedure started on	01 December 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	20 February 2023
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	06 March 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	03 March 2023
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	30 March 2023
The following GCP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection at two clinical sites, one in Germany, one in France and the sponsor in the USA were conducted between 20 Feb and 31 Mar 2023. The outcome of the inspection carried out was issued on 05 June 2023.	05 June 2023
The applicant submitted the responses to the CHMP consolidated List of Questions on	13 July 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	21 August 2023
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	31 August 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	14 September 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	09 October 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	25 October 2023
SAG/Expert group (were convened to address questions raised by the CHMP on The CHMP considered the views of the SAG/Expert group as presented in the minutes of this meeting.	30 October 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	09 November 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	14 December 2024

The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	10 January 2024
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 Qalsody on	22 February 2024
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	22 February 2024
The European Commission returned the Opinion to the Agency, requesting to further substantiate the recommendation to grant a marketing authorisation under exceptional circumstances for Qalsody	22 March 2024
The CHMP, in the light of the available scientific data and scientific argumentations, considered that the conditions for authorisation under exceptional circumstances are fulfilled and adopted a revised positive Opinion recommending the granting of the marketing authorisation.	19 April 2024

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Amyotrophic lateral sclerosis (ALS), first described by Charcot in 19th century, (also known as "Lou Gehrig's disease") is a rare, progressive, and ultimately fatal neurodegenerative disease that causes loss of upper and lower motor neurons and their axons within the cortex, brainstem, spinal cord, and peripheral nervous system. The loss of motor neurons leads to progressive loss of muscle mass, strength, and function in bulbar, respiratory, and limb muscles, typically leading to paralysis and ultimately death from respiratory failure within approximately 3 years of symptom onset (with medians from different studies ranging from 1.6 to 5.2 years). Long survivors (defined as >8 years from diagnosis) have also been reported recently in a cohort in Scotland (Leighton et al 2022). Disease progression for individual mutations is variable, with survival ranging from less than a year to over 20 years (Bali 2017; Cudkowicz 1997).

ALS may present in any anatomical region and spread throughout the body with variable speeds and patterns in different patients. ALS is a disease with limited treatment options.

Functional decline and death are inevitable unless tracheostomy and mechanical ventilation are elected to support life, although these interventions do not stop or slow the decline in physical independence.

SOD1-ALS is a primarily autosomal-dominant disorder representing approximately 2% of the ALS population. In these patients, mutations in SOD1 gene lead to accumulation of a toxic form of SOD1 protein. The natural history of SOD1-ALS is highly variable with a mean onset of 49.7 ± 12.3 years and mean disease duration of 4.6 (1.4 for A4V and 6.6 for non-A4V) years.

The target indication is: Qalsody is indicated for the treatment of adults with amyotrophic lateral sclerosis (ALS), associated with a mutation in the superoxide dismutase 1 (SOD1) gene.

2.1.2. Epidemiology and risk factors

ALS is a rare disease. The global prevalence of ALS is estimated to be approximately 4.42 per 100,000.

ALS has historically been classified as familial (fALS; 5% to 10% of ALS cases) or sporadic (sALS; approximately 90% to 95% of ALS cases) based on the presence or absence of a known family history. However, both fALS (approximately 70%) and sALS (approximately 10%) can be caused by genetic mutations. This is thought to be attributable, at least in part, to certain limitations, including the lack of a standardized definition of what constitutes a family history, challenges with obtaining a complete and accurate family history, and multiple reasons why a family history of ALS could be missed during diagnosis (e.g., small family size or reduced penetrance).

In approximately 2% of people living with ALS, the disease is attributed to mutation(s) in the gene encoding the enzyme SOD1. Applying the pooled ALS incidence and prevalence rate estimates for Europe to an additional 15 European countries, the estimated numbers are 314 incident and 844 prevalent SOD1-ALS cases across 37 European countries. Based on these estimates, the prevalence and incidence rates of SOD1-ALS in Europe can be calculated as 0.12 and 0.04, respectively.

2.1.3. Aetiology and pathogenesis

The aetiology of ALS is unknown. A number of potential mechanisms have been proposed including abnormal RNA processing, disorders of protein quality control, excitotoxicity, cytoskeletal derangements, mitochondrial dysfunction, viral infections, apoptosis, growth factor abnormalities, inflammatory responses, and others.

The disease is attributed to mutation(s) in the gene encoding the enzyme SOD1. Over 200 SOD1 mutations associated with ALS have been identified to date, with varying degrees of evidence about pathogenicity. All SOD1 variants associated with ALS (> 200 identified to date) are assumed to cause SOD1 gain of function; i.e., they lead to an accumulation of mutant SOD1 protein that is believed to underlie the pathogenicity of SOD1 ALS. These mutations primarily follow an autosomal-dominant inheritance pattern, though the p.Asp91Ala (D91A; D90A) mutation most prevalent in the Scandinavian population also displays recessive inheritance. The SOD1 gene encodes an abundant dimeric enzyme, copper/zinc superoxide dismutase (Cu/Zn SOD or SOD1), which catalyses the transmutation of superoxide (O2-) into oxygen (O2) and hydrogen peroxide (H2O2).

ALS is characterized by motor neuron degeneration and death with gliosis replacing lost neurons. Cortical motor cells (pyramidal and Betz cells) disappear, leading to retrograde axonal loss and gliosis in the corticospinal tract. This gliosis results in the bilateral white matter changes sometimes seen in the brain magnetic resonance imaging (MRI) of patients with ALS. The spinal cord becomes atrophic. The ventral roots become thin, and there is a loss of large, myelinated fibers in motor nerves. The affected muscles show denervation atrophy with evidence of reinnervation such as fiber type grouping.

2.1.4. Clinical presentation, diagnosis and prognosis

The loss of motor neurons results in the primary clinical symptoms and signs of ALS. These may produce impairment affecting limb, bulbar, axial, and respiratory function.

The clinical hallmark of ALS is the combination of upper and lower motor neuron signs and symptoms. The upper motor neuron findings of weakness with slowness, hyperreflexia, and spasticity result from degeneration of frontal lobe motor neurons located in the motor strip (Brodmann area 4) and their axons traversing the corona radiata, internal capsule, cerebral peduncles, pontine base, medullary pyramids, and the lateral corticospinal tracts of the spinal cord. The lower motor neuron findings of weakness,

atrophy or amyotrophy, and fasciculations are a direct consequence of degeneration of lower motor neurons in the brainstem and spinal cord producing muscle denervation.

The natural history of SOD1-ALS is highly variable and generally poorly characterized due to the rarity of the disease and limited data availability. The rapidity of disease progression also varies substantially across SOD1 mutation types.

2.1.5. Management

There is a high unmet medical need for effective therapies that preserve function and prolong life for people living with ALS. Functional decline and death are inevitable unless tracheostomy and mechanical ventilation are elected to support life, although these interventions do not stop or slow the decline in physical independence. Symptomatic management includes treatment options for pain, cramps, excess saliva, spasticity, fatigue, pseudobulbar affect, anxiety, and insomnia; therapy (physical, occupational, and speech); and ventilation assistance.

Riluzole, the most widely approved ALS treatment, received its first global approval for treatment of ALS in 1995 (US) based on 2 studies that demonstrated that the time to tracheostomy or death was approximately 2 months longer in participants receiving riluzole than in those receiving placebo.

Edaravone received its first approval for the treatment of ALS in 2015 in Japan based on a single study in Japanese patients with ALS of Grade 1 or 2 in the Japan ALS Severity Classification. In Europe, a MA application was filed (EMEA/H/C/004938) and later withdrawn in 2019. Edaravone (Radicava) is not approved in the EU. Edaravone is available through a compassionate use programme in some EU countries.

Sodium phenylbutyrate/ursodoxicoltaurine (AMX0035 also called taurusodiol; tradename Albrioza and Relyvrio) was recently approved in Canada and the US for the treatment of ALS. In EU, a MA application was filed (EMEA/H/C/005901) but received a final negative opinion on 12 October 2023.

2.2. About the product

Tofersen is a 20-base residue (20-mer) ASO that contains 15 phosphorothioate diester and 4 phosphate diester linkages.

Tofersen is complementary to a portion of the 3’ untranslated region of the messenger ribonucleic acid (mRNA) for human SOD1, binding by Watson-Crick base pairing (hybridisation). This hybridisation of tofersen to the cognate mRNA results in RNase-H-mediated degradation of the mRNA for SOD1, which reduces the amount of SOD1 protein synthesis and accumulation.

The recommended dose is 100 mg of tofersen per treatment.

Tofersen treatment should be initiated with 3 loading doses administered at 14-day intervals.

A maintenance dose should be administered once every 28 days thereafter.

Qalsody is administered as an intrathecal bolus injection using a lumbar puncture needle over 1 to 3 minutes.

2.3. Type of Application and aspects on development

The applicant requested consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation based on inability to

provide comprehensive efficacy and safety data due to rarity of the indication. The SOD1-ALS is an ultra-rare condition. The applicant estimates that there are less than 1000 prevalent cases in the EU. It is pointed out that additional placebo-controlled studies in symptomatic SOD1 ALS are not considered operationally feasible. Thus, the applicant considers that it will not be possible to provide comprehensive data on the efficacy and safety under normal conditions of use.

2.4. Quality aspects

The finished product is presented as solution for intrathecal injection containing 100 mg/15 mL of tofersen as active substance. Each ml contains 6.7 mg of tofersen.

Other ingredients are sodium dihydrogen phosphate dihydrate, disodium phosphate, sodium chloride, potassium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, and water for injection.

The product is available in a 20 ml Type I glass vial closed with a chlorobutyl rubber stopper and aluminium overseal with a flip-off plastic button.

2.4.1. Active Substance

2.4.1.1. General Information

Tofersen is a 20-base residue (20-mer) 5-10-5 MOE gapmer mixed backbone oligonucleotide. Of the nineteen internucleotide linkages, fifteen are 3′-O to 5′-O phosphorothioate diesters, and four are 3′-O to 5′-O phosphate diesters. Ten of the twenty sugar residues are 2′-deoxy-D-ribose and the remainder are 2′-O-(2-methoxyethyl)-D-ribose (MOE). The residues are arranged so that there are five MOE nucleosides at the 5′ and 3′-ends of the molecule flanking a gap of ten 2′- deoxynucleosides.

The chemical name of tofersen is *2′-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-adenylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-P-thioguanylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-guanylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-P-thioadenylyl-(3′-O→5′-O)-2′-deoxy-P-thiothymidylyl-(3′-O→5′-O)-2′-deoxy-P-thioadenylyl-(3′-O→5′-O)-2′-deoxy-5-methyl-P-thiocytidylyl-(3′-O→5′-O)-2′-deoxy-P-thioadenylyl-(3′-O→5′-O)-2′-deoxy-P-thiothymidylyl-(3′-O→5′-O)-2′-deoxy-P-thiothymidylyl-(3′-O→5′-O)-2′-deoxy-P-thiothymidylyl-(3′-O→5′-O)-2′-deoxy-5-methyl-P-thiocytidylyl-(3′-O→5′-O)-2′-deoxy-P-thiothymidylyl-(3′-O→5′-O)-2′-deoxy-P-thioadenylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-5-methylcytidylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-P-thioadenylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-guanylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3′-O→5′-O)-2′-O-(2-methoxyethyl)-5-methyluridine* corresponding to the molecular formula $C_{230}H_{317}N_{72}O_{123}P_{19}S_{15}$ (free acid).

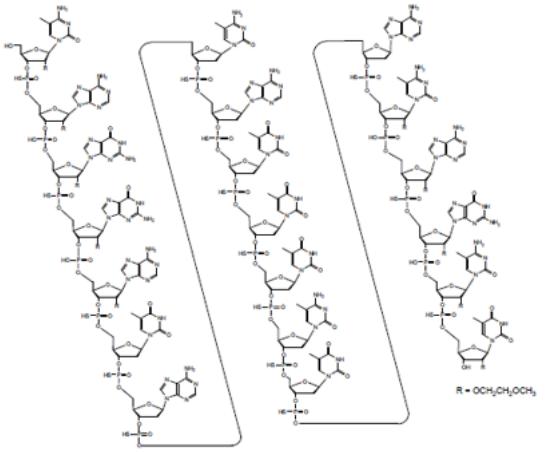
The tofersen sequence can be written in shorthand as follows:

5′-^{Me}CsAoGsGoAsTsAs^{Me}CsAsTsTsTs^{Me}CsTsAs^{Me}CoAsGo^{Me}Cs^{Me}U-3′

The underlined residues are MOE nucleosides. The locations of phosphorothioate and phosphate diester linkages are designated by s and o, respectively.

It has a relative molecular mass of 7127.86 g/mol and the following structure (Figure 1):

Figure 1: active substance structure

Chemical Structure	
Molecular Formula	$C_{230}H_{317}N_{72}O_{123}P_{19}S_{15}$ (free acid)
Molar Mass	7127.86 amu

The chemical structure of tofersen was elucidated by a combination of ion-pair high performance liquid chromatography, 1H-/13C-/31P-NMR, TOF-MS, LC-TOF-MS, MS/MS, Tm ,CD and UV spectroscopy. NMR results were consistent with the expectations for the active substance structure. Also, accurate mass, sequence analysis and melting temperature indicate the correct primary structure.

Tofersen exhibits stereoisomerism due to the presence of 15 chiral centres. Tofersen is a mixture of 2^{15} (i.e. 32,768) diastereoisomers. From the characterisation data, diastereomer distribution calculations were performed.

The solid-state properties of the active substance are not considered relevant, as the tofersen active substance is formulated as a liquid consisting of tofersen in solution with sodium phosphate monobasic dihydrate, sodium phosphate dibasic anhydrous, and sodium chloride.

2.4.1.2. Manufacture, process controls and characterisation

The active substance is manufactured by one manufacturing site.

Tofersen is a synthetic oligonucleotide that is manufactured in a 7-step process, using well defined starting materials with acceptable specifications.

Solid phase synthesis is performed on an oligonucleotide synthesizer. Synthesis is followed by cleavage of the oligonucleotide from the solid support and removal of the amide protecting groups from the nucleobases. Purification steps follow. Ultimately the active substance is, after filtration through a sterilisation grade filter, dispensed and stored in bags, protected from light. The narrative process description covers applied reagents, process conditions, tabular overviews on critical controlled parameters as well as critical in-process controls/tests including limits and is sufficiently detailed.

The proposed in process controls and the acceptable ranges for the operation of critical steps in the tofersen active substance manufacturing process have been provided. These controls have been established to ensure that the process produces acceptable product quality.

The starting materials for the manufacture of tofersen are defined. The choice of starting materials is well justified according to the principles outlined in ICH Q11. The applicant confirms that upon change or addition of starting material suppliers a variation will be submitted.

Detailed information on critical (result in impurities that cannot be removed during purifications steps) and non-critical (have no negative impact on drug substance purity) impurities in the phosphoramidites

is provided. Structure, origin, prevalence and resulting drug substance impurities are described. The starting material specifications are defined. The starting materials specifications are sufficiently justified. Summaries of historical batch data are provided and well within the currently proposed specifications.

It is confirmed that no materials of biological origin are used in the commercial manufacturing process.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Changes introduced have been presented in sufficient detail and have been justified.

The process development history, namely changes in process and the respective rationales, is well described. Batch data is provided from before and after the different changes to show comparability sufficiently. The overall control strategy is described The applicant indicates that a risk-based approach based on ICH Q8 and ICH Q9 was applied throughout the product development lifecycle. In a product risk assessment, based on a tofersen quality target product profile, active substance, and finished product CQAs and non-CQAs were identified. The classifications and justifications are acceptable.

Manufacturing development studies were performed to assess the impact of process performance on the product quality attributes. These were confirmed in process validation. Sufficient details have been provided on Process Risk Assessment to comprehend the respective results. Extensive process characterisation studies have been performed to optimise the commercial process. The proposed overall control strategy could be acceptable.

Finally, it should be pointed out that the active substance is a solution. The addition of the formulation electrolyte excipients (KCl, $CaCl_2$, $MgCl_2$) and final dilution to target concentration were relocated from the active substance to the finished product process. Elimination of the lyophilisation step is considered justified. The overall strategy regarding the liquid drug substance is acceptable.

The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

Impurities are classified into organic impurities, inorganic impurities and residual solvents. Inorganic impurities, residual solvents, small molecule impurities, impurities with genotoxic potential and nitrosamines are discussed.

The organic impurities are sub-classified into process-related impurities, impurities derived from starting materials and reagents as well as potential degradation products. The grouping strategy for the organic impurities is based on their structures and formation mechanisms and well justified by toxicological qualification of the impurity groups. This is acceptable for a synthetic oligonucleotide with a complex impurity profile. A good general understanding of possible organic impurities is demonstrated. Impurity characterisation was performed. It is justified that the test method is suitable and sufficient for characterisation of all impurities and degradation products in the active substance. The active substance in solution is packaged in sterile, single use, flexible bioprocess bags with a LDPE contact layer. The bags are sealed with polycarbonate aseptic connectors. An acceptable specification for the primary container closure is provided. The applicant indicates compliance with the European Pharmacopoeia and foodstuff legislation (European Union Directive 2011/10/EC Regarding Plastic Materials and Articles Intended to Come into Contact with Food). Extractable studies were performed with model solvents on the inner bag material. An adequate evaluation of the safety risks of measured extractables concluded that levels of identified extractables are all below accepted toxicity limits.

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

The active substance specification includes tests for appearance (visual), pH (Ph. Eur.), concentration (LC-UV-MS), identity (LC-UV-MS, LC-UV, Failure Sequence Analysis), purity and impurities (LC-UV-MS), bacterial endotoxins (Ph. Eur.) and bioburden (Ph. Eur.).

The proposed specification is acceptable and appropriate limits have been set in line with guidelines, batch data from development, clinical and stability batches.

Impurities are grouped according to their structures and formation mechanisms. The concept of grouping as proposed in the specification is acceptable. All relevant active substance attributes are covered and omission of residual solvents and elemental impurities testing is justified. Process-related impurities are adequately controlled.

The selected set of impurities is acceptable. The specification limits for the specified impurities in the active substance are established based on historical release and stability data in addition to toxicological qualification data. Identification and qualification thresholds are acceptable. A risk assessment indicated that levels of elemental impurities are below the control threshold. Omission of routine testing for elemental impurities is therefore acceptable. A nitrosamine risk assessment was performed, and the report included in Module 1. It was concluded that there is no risk of presence of nitrosamine impurities in the drug substance. No confirmatory testing was performed. The conclusions are reasonable. The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for release testing has been presented.

Batch analysis data (13 batches) of the active substance are provided comprising release data for nine non-clinical toxicology/clinical/stability batches/process development batches as well as for four process validation/commercial/stability batches is included in the dossier. Purity/impurity data for five clinical or pilot batches is provided additionally. The proposed commercial specifications or historic specifications are met for all attributes in all batches.

2.4.1.4. Stability

Stability studies were conducted in accordance with ICH Q1A (R2) and data for four primary process validation batches produced by the commercial process are provided. Data were collected at the long-term condition of 5±3 °C/Ambient RH for 24 months and at the accelerated condition of 25 °C ± 2 °C/60±5% RH for 6 months for all batches. Long-term stability studies are ongoing. The active substance is stored in sterile bags with PE product contact layer representative for the commercial container closure system during stability studies. Stability protocols covering 60 months at long-term and 6 months at accelerated conditions comprising appearance, pH, purity, impurities and concentration by LC-UC-MS are provided.

Most attributes did not change during 24 months at long-term or 6 months at accelerated conditions. All results remain within specifications. Statistical analyses indicate that specifications will be met throughout the proposed retest period. No details are provided on methodology and results of said statistical analysis. This is acceptable since no retest period extrapolation is performed.

The stability data for all batches and both storage conditions show variability over time. Since the values do not present any trend and are well below proposed specifications limits, this is acceptable.

Forced degradation studies have been performed for the following stress types: visible light, ultraviolet light, acid, alkaline, oxidative, heat. Representative chromatograms including peak assignment are

provided. Two main degradation products were detected under the most relevant stress condition of heat.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 24 months when stored at 5±3 °C/Ambient RH in the proposed container. The post-approval stability protocol and stability commitment are acceptable.

2.4.2. Finished Medicinal Product

2.4.2.1. Description of the product and Pharmaceutical Development

The finished product is an aqueous solution for intrathecal injection. It is presented as clear and colourless to slightly yellow solution with a pH of 6.7 to 7.7. The finished product is supplied in a Type I glass vial closed with a rubber stopper and seal with a flip-off cap. Each vial of finished product contains a single dose of 100 mg tofersen at a concentration of 6.7 mg/mL in a formulation at pH 7.2.

The quantitative composition, function, and quality standard of each component in the finished product are provided. There is sufficient fill to ensure a 15.0 mL deliverable volume. There are no overages in the drug product formulation.

Tofersen finished product was designed as a sterile solution for intrathecal injection in a single-dose glass vial with a rubber stopper. A comprehensive QTPP has been established. The formulation and the manufacturing process were developed based on the CQAs required for this type of finished product.

The active substance is in a buffered aqueous solution. The properties of the active substance that may affect the finished product manufacturing process are concentration of ASO, concentration of sodium phosphate/sodium chloride and pH.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

For intrathecally applied finished product, the composition corresponding to endogenous cerebrospinal fluid with physiological pH and osmolality is critical. Tofersen was found to be physically and chemically stable in the artificial cerebrospinal fluid (aCSF). Different tofersen concentrations, freeze-thaw stability and robustness to differences in excipients concentrations and pH were investigated. No significant impact on purity and impurities was found.

For clinical supply the product was initially provided as a concentrate in aCSF together with a second vial containing aCSF diluent ("Two-vial presentation") for dose selection. The selected dose for pivotal studies was 100 mg, provided as a ready-to-use (RTU) solution for injection. The excipients are the same between the two-vial and the RTU presentations. The commercial formulation is the same as the 100 mg RTU formulation used in the pivotal clinical studies. The finished product does not contain overages. However, an overfill is necessary to extract the nominal volume of 15 mL from the vials. The limits are acceptable, as per USP <1151>.

The manufacturing process is a common process for aqueous sterile finished product that cannot be subjected to terminal sterilisation. It consists of electrolyte solution and aCSF dilution buffer preparation, finished product bulk compounding, bioburden reduction filtration, in-line sterile filtration, aseptic vial filling, stoppering and capping followed by visual inspection.

The applicant claims that steam sterilisation of the finished product is not feasible. Although the degradation of the active substance is not considered significant, this is acceptable due to the incompatibility of the aCSF for terminal sterilisation demonstrated with an autoclave cycle of F_0=8 min. Due to the concentration of salts, precipitation occurs in the solution (turbidity).

The development of the manufacturing process is described. A list of process steps with their respective process controls and criticality classification is provided, which is considered adequate. Comparability of the commercial manufacturing process to the previous processes used for clinical supply was demonstrated based on release and stability data.

The applicant provided conclusions of risk assessments concerning the contribution of the product and manufacturing components to the levels of endotoxins in the finished product, extractables and leachables from process wetted surfaces, and elemental impurities in the finished product. With respect to extractables and leachables from process contact surfaces no risks to patient safety have been identified.

The commercial primary container closure system (CCS) for the finished consists of type 1 clear glass vials closed with chlorobutyl rubber stoppers with cross-linked silicone oil coating.

Sufficient information has been provided for the CCS in terms of protection, security and compatibility. For the quality requirements of the type I glass and the chlorobutyl rubber of the packaging components reference is made to the relevant compendial monographs. Additional tests carried out by the applicant cover supplier certificate check, catalog number check, appearance, and dimensions.

Container closure integrity testing results demonstrate that the primary container closure components prevent microbial ingress under the foreseen storage conditions. Leachable data showed no leachables above the Analytical Evaluation Threshold (0.1 µg/mL) derived from the Threshold of Toxicological Concern (ICH M7: ≤ 1.5 µ g/day). The chemical compatibility of the CCS with the finished product has been demonstrated in stability studies.

The compatibility of the finished product with a polypropylene syringe for withdrawing the solution from the vials, a stainless steel/polypropylene spinal needle, and a polypropylene IV extension set was tested. This in-use test supports the instructions provided in the SmPC section *4.2 Posology and method of administration*: "Once drawn into the syringe, the solution should be administered immediately (within 4 hours since removal from refrigeration) at room temperature; otherwise, it must be discarded."

2.4.2.2. Manufacture of the product and process controls

The finished product is manufactured by one manufacturing site. Biogen Netherlands B.V., Prins Mauritslaan 13, 1171 LP Badhoevedorp, The Netherlands is responsible for batch release in the EU/EEA. The finished product manufacturing process covers a batch size range and comprises four steps plus a pre-processing preparation of electrolyte solution and aCSF dilution buffer. The manufacturing process starts with compounding of bulk drug product including bioburden reduction filtration in-line sterile-filtration, aseptic vial filling, stoppering and capping , and visual inspection . The process is considered to be a non-standard manufacturing process.

The applicant defines a reprocessing step. This reprocessing step was part of the PPQ and is considered acceptable.

Critical process steps and the corresponding critical process parameters (CPPs) and their action limits were presented. Appropriate in-process controls for the various process steps were also presented.

Major steps of the manufacturing process have been validated by a number of studies. In terms of IPCs and the release testing results, all acceptance criteria for the PPQ batches were met. In addition, media

fill runs demonstrated that the filling conditions are validated. The bracketing approach is accepted given the straightforward manufacturing process. Validation studies were performed on the membrane used for sterile filtration.

It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

2.4.2.3. Product specification, analytical procedures, batch analysis

The finished product specification includes tests for appearance (visual), pH (Ph. Eur.), osmolality (Ph. Eur.), assay (LC-UV), extractable volume (Ph. Eur.), identity (LC-UV, melting temperature), purity (LC-UV), degradation products (LC-UV), particulate matter (Ph. Eur.), bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), container closure integrity testing (High Voltage Leak Detection).

The proposed specification is acceptable and appropriate limits have been set in line with guidelines, batch data of clinical batches, stability data, manufacturing capability and variability and analytical test method capability.

Based on release and stability data, the applicant proposes specification limits for % label claim and to review the assay limit through annual review when more DP lots are produced. This is acceptable. The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for release and stability testing has been presented. They are the same as those used for active substance testing.

The risk assessment and confirmatory testing of four finished product batches confirm that elemental impurities are consistently below the PDE from ICH guideline Q3D (R2) on elemental impurities. Therefore, routine controls are not required.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed (included in Module 1) 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 of the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Release results for development batches manufactured by earlier process(es) and for batches manufactured by the proposed commercial process are provided. Based on the batch results it can be concluded that the manufacturing process is under control and results in consistent and uniform drug product.

2.4.2.4. Stability of the product

Stability studies in accordance with ICH Guideline Q1A for the four PPQ batches were conducted. In addition, stability studies for supportive finished product (7 batches) manufactured during process development and for clinical use are presented. Samples have been stored at the long-term (5 ± 3°C/Ambient RH), accelerated (25 ± 2°C/60 ± 5%RH) and stressed storage conditions (40 ± 2°C/75 ± 5%RH). The stability study protocols are provided.

The analytical methods for stability testing of batches and the clinical/process development batches are the same as those used for release testing. Finished product was stored in containers that are representative of the commercial containers.

For the PPQ batches) long-term stability data are available for 24 months, for the clinical/development batches for up to 48 months. Stability results at accelerated conditions are presented for 12 months and at stress conditions for 6 months. Under long-term storage conditions, no significant changes were observed in any of the attributes. Under accelerated conditions, impurities increase slightly, under stress conditions they are more pronounced after 6 months.

Photostability studies demonstrated that the drug product needs to be kept in the secondary packaging as the finished product is sensitive to light exposure. However, further studies demonstrated that the finished product can be held for 36 hours at room temperature and ambient light following removal from the secondary packaging.

An additional study showed that the finished product in its secondary packaging can be held at room temperature not exceeding 30 °C for up to 2 weeks within the drug product shelf-life.

Thermal cycling studies confirmed that short-term temperature excursions during supply chain handling have no negative impact on drug product quality.

Based on available stability data, the proposed shelf life and storage conditions stated in the SmPC (section 6.3 and 6.4) are acceptable:

Shelf life: *30 months*

The vial of QALSODY in its original carton can be stored for up to 14 days at room temperature (not to exceed 30°C).
Unopened vials of QALSODY can be removed from and returned to the refrigerator, if necessary. Unopened vials can be removed from the original carton for not more than 6 hours per day at room temperature for a maximum of 6 days.

Storage conditions:
Store in a refrigerator (2°C - 8°C).
Do not freeze.
Store in original package in order to protect from light."

A sufficient post-approval stability protocol and commitment has been included.

2.4.2.5. Post approval change management protocol(s)

Not applicable.

2.4.2.6. Adventitious agents

No materials of animal or human origin are used in the manufacture of the active substance or finished product.

2.4.3. Discussion on chemical and pharmaceutical 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.

2.4.4. 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.

2.4.5. Recommendation(s) for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Introduction

The non-clinical program with tofersen was conducted using *in vitro* and *in vivo* studies to support chronic intrathecal (IT) administration for the treatment of ALS associated with a mutation in the SOD1 gene. The non-clinical testing strategy for tofersen followed a development pathway typical of a pharmaceutical product and was consistent with existing regulatory guidance (ICH M3(R2)). The objectives of the non-clinical program were to evaluate the pharmacology, pharmacokinetics (PK), and safety of tofersen as follows:

- Pharmacology studies to evaluate the effect of SOD1 lowering and efficacy *in vitro* and in both transgenic mouse and rat models as well as non-human primates (nonhuman primate NHPs, Cynomolgus monkeys),
- PK studies to evaluate the half-life of tofersen in central nervous system (CNS) tissue to aid in estimating IT dosing frequency in patients and correlate antisense oligonucleotide (ASO) cerebrospinal fluid (CSF) concentrations with tissue concentrations in animal pharmacology and toxicology models. Furthermore, the PK profile of tofersen was characterized in plasma of animals. Additional studies were conducted to characterize the potential for drug-drug interactions (DDI), including protein binding, metabolite identification, inhibition, or induction of CYP450 enzymes, and inhibition and binding to absorptive and efflux transporters,
- GLP non-clinical safety pharmacology and toxicology studies to support the chronic use of tofersen for the treatment of patients with ALS.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Tofersen is complementary to a portion of the 3’ UTR of the mRNA for human SOD1. The binding of tofersen to the cognate mRNA results in RNase-H1-mediated degradation of the mRNA for SOD1, and thus reduces the amount of SOD1 protein. RNase H1 is a ubiquitously expressed nuclease that recognizes a DNA-RNA heteroduplex and cleaves the RNA strand of the duplex. Since tofersen binds to a site in the 3’ UTR of human SOD1 mRNA, it is expected to reduce expression of both wild type and mutant variants of SOD1.

Primary PD of tofersen was studied *in vitro* and *in vivo*. A series of informatics searches were completed to assess the potential cross-reactivity in non-human species and polymorphisms in the target-binding site of the SOD1 gene in the general human population and specifically in ALS mutation databases were examined. The analyses support presence of the tofersen binding site in the target patient population. In addition, no relevant polymorphisms were identified in the SOD1 gene of ALS patients. Furthermore, a single base-pair mismatch to the Cynomolgus monkey gene suggests it may be active in NHPs, thereby enabling assessment of possible on-target effects, while wild-type animals of all other species appear not to be suitable due to at least four or more mismatches in the target sequence.

The *in vitro* potency of tofersen was established in two human cell lines (SH-SY-5Y and A431) and monkey hepatocytes. Tofersen suppressed SOD1 mRNA in a concentration-dependent manner in all cell lines (half-maximal inhibitory concentration (IC_{50}): 1.1 µM, 0.65 µM and 0.98 µM in SH-SY5Y, A431 cells and monkey hepatocytes, respectively).

In vivo activity of tofersen was investigated in SOD1-G93A mice and rats and in monkeys. An overview of performed studies and relevant findings are displayed in Table 1.

*Table 1: Overview of in vivo PD studies performed with tofersen***.**

Study Number	Study Type	Test System	Results / ED_{50} / EC_{50}
EXP-15-AA2184	Dose-response evaluation of tofersen in human SOD1-G93A mice	SOD1-G93A mice (6-8 weeks old), 10, 30, 100, 300, or 700 µg tofersen ICV single dose, sample collection at 2 weeks post dose	**SOD1 mRNA reduction, ED_{50}:** Lumbar cord: 64 µg Cervical cord: 44 µg Cortex: 144 µg **SOD1 mRNA reduction per µg tofersen/g tissue, EC_{50}:** Lumbar cord: 0.87 µg/g Cortex: 7.9 µg/g
ASO-13	Survival study to assess effect of time of tofersen injection on survival of human SOD1-G93A mice	SOD1-G93A mice, 300 µg tofersen or control ASO ICV at 30, 50, 80, or 110 days of age, single dose	**Median disease onset at dosing at 30, 50, 80, or 110 days of age:** Control ASO: 147.5, 151, 145, and 155 days, respectively Tofersen: 168, 169, 176.5, and 188 days, respectively. **Median survival at 30, 50, 80, or 110 days of age:** Control ASO: 170, 162, 164, and 167 days, respectively Tofersen: 193.5, 182, 186.5, and 201 days, respectively.
ASO-14	Effect on survival and motor performance of 2 different doses of tofersen in human SOD1-G93 mice	SOD1-G93A mice, 100 µg, 300 µg tofersen or 300 µg control ASO ICV at 50 and 94 days of age	**Disease onset:** Control ASO = 20 weeks; tofersen 100 µg = 25.3 weeks; tofersen 300 µg = 26.1 weeks **Median survival:** control ASO = 24 weeks; tofersen 100 µg = 28.3; tofersen 300 µg = 29.3 weeks **Rotarod performance:** Tofersen 100 µg and 300 µg: comparable improvements on motor performance. Tofersen-treated animals stayed on the rotarod

			significantly longer relative to control ASO treated mice.
ASO-15	Electrophysiological and histological study on the effect of tofersen in human SOD1-G93A mice	SOD1-G93A mice, 300 µg tofersen or control ASO ICV single dose at 35 days of age, sample collection at weeks 15 and 17 post dose	Tofersen significantly protected the neuromuscular structure and function and reversed effects comparable to wild-type mice.
ASO-27	Histological study on the effect of tofersen in human SOD1-G93 mice	SOD1-G93A mice, 100 µg tofersen or control ASO ICV at 50 and 94 days of age, sample collection at 16- and 24-weeks post dose	Tofersen significantly protected denervation-dependent fiber-type switching in the anterior tibialis muscle. Moreover, tofersen significantly decreased the amount of microglial activation in the spinal cord, suggesting protective activity against inflammation in the CNS.
ASO-28	Analysis of dose-response efficacy of tofersen in human SOD1-G93A mice	SOD1-G93A mice, 10 µg, 30 µg, 100 µg tofersen or 100 µg control ASO ICV at day 35 of age, sample collection at days 56 (serum) and 70 (serum/tissues).	**Native SOD1 protein levels:** Significant reduction in cortex and spinal cord at ≥ 10 µg **Misfolded SOD1 protein levels:** Significant reduction in cortex at ≥ 10 µg and spinal cord at 100 µg only **Tibialis anterior muscle function:** Significant effect at ≥ 30 µg **pNfH serum levels:** Significant reduction at ≥ 30 µg at 8 and 10 weeks
EXP-15-AA2185	Dose-response evaluation of tofersen in human SOD1-G93A rats	SOD1-G93A rats, 10, 30, 100, 300, 1000, or 3000 µg tofersen, IT single dose, sample collection at 2 weeks post dose	**SOD1 mRNA reduction, ED_{50}:** Lumbar cord: 48 µg Cervical cord: 93 µg Cortex: 534 µg **SOD1 mRNA reduction per µg tofersen/g tissue, EC_{50}:** Lumbar cord: 1.4 µg/g Cortex: 2.3 µg/g
EXP-15-AA2186	Time course of *in vivo* efficacy (RNA and protein levels) of tofersen in human SOD1-G93A rats	SOD1-G93A rats, 1000, 3000 µg IT twice, ICV 1000 µg single dose, tissue collection at 2, 4 and 8 weeks post dose	Tofersen lowered both SOD1 mRNA (> 50%) and protein in lumbar cord, cervical cord and cortex. Furthermore, these tissue changes are reflected as changes in SOD1 protein in CSF (> 50% at weeks 4 and 8).
666853-AS01	Dose-response evaluation of tofersen in Cynomolgus monkeys as part of a 13 weeks repeat-dose toxicity study	Cynomolgus monkeys, 4, 12, 35 mg IT by lumbar bolus injection biweekly for the first month (Days 1, 14, and 28), then monthly thereafter (Days 56 and 84). Sample	**SOD1 mRNA reduction per µg tofersen/g tissue, EC_{50}:** Brain tissues (combined): 20.7 µg/g

	13-weeks with a 13-week recovery phase	collection 1 week after last dose	

In order to establish *in vivo* potency, C57BL6 mice expressing the human SOD1 locus, including the 3' UTR to which tofersen is targeted, were used as main animal model (Gurney, 1994). This transgenic mouse model was chosen because the binding site of tofersen is not conserved in wild-type mouse or rats.

Tofersen showed suppression of SOD1 mRNA in SOD1-G93A mice, with a half-maximal effective concentration (EC_{50}) of 0.87 µg/g in the lumbar spinal cord and 7.9 µg/g in the cerebral cortex. Based on the EC_{50} of 0.87 µg/g in the target tissue spinal cord, a target exposure for patients of ~ 1 µg/g tissue was predicted and this exposure is anticipated to be reached in patients. Autopsy samples of three participants showed tofersen tissue concentrations of > 200 µg/g in the target spinal cord regions.

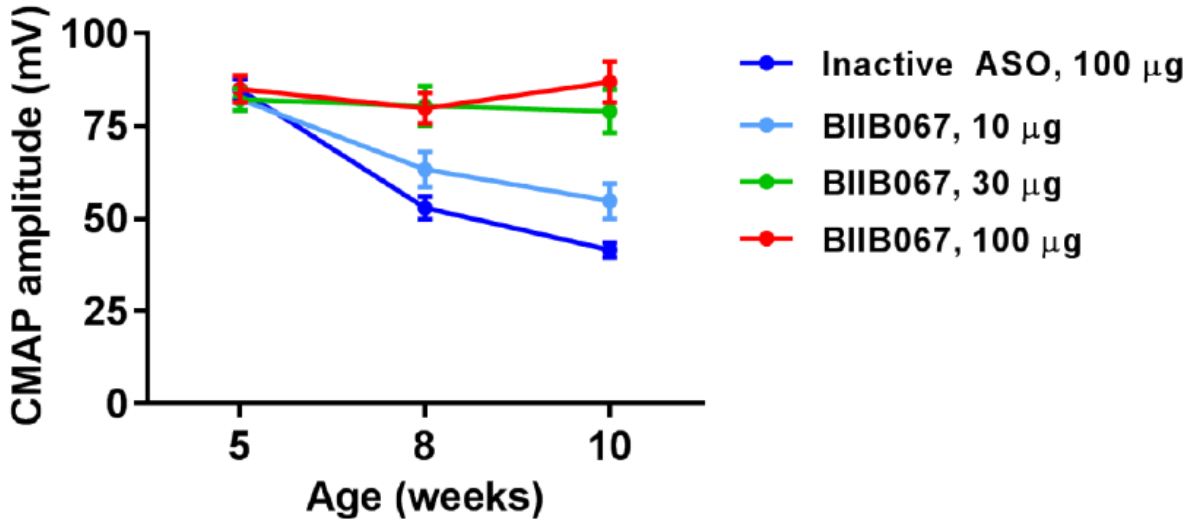
Dosing of SOD1-G93A mice with tofersen administered by intracerebroventricular (ICV) injection in the range of 30 µg – 300 µg also significantly delayed disease onset (reduction in body weight) and survival for all treatment ages, improved motor performance, prevented myofiber atrophy as measured by myofiber diameter in the tibialis anterior and improved fiber type composition of the soleus muscle loss of muscle function. In addition, tofersen reduced levels of neuroinflammation markers in the spinal cord of SOD1-G93A mice and treatment dose-dependently lowered serum levels of phosphoneurofilament heavy chain (pNfH).

The mice were 35 days old at the start of therapy, which corresponds to a human age of about 3.8 years (assuming 1 human year corresponds to 9 mouse days). Afterwards, they were examined for another 35 days, corresponding to 3.8 human years (Day 70, Week 10). First effects on pNfH were seen after 8 weeks (measurements before that were not possible), which corresponds to 2.3 human years of treatment (Das 35 to Day 56 = 21 days = 2.3 human years).

The animals were treated with a single dose of ICV injection, first effects appeared at 30 µg/mouse. The half-maximal effective dose (ED_{50}) for the reduction of SOD1 mRNA is in the range of 64 µg/mouse in the lumbar cord, which corresponds to 0.87 µg/g tissue lumbar cord. In humans > 200 µg/g were measured in the spinal cord in 3 volunteers. Assuming that a mouse weighs approx. 20 g, the dose would be 30 µg x 50 = 1.5 mg/kg, i.e. 105 mg for a 70 kg adult, which is almost exactly the human dose.

In the same experiment, the muscle function (tibialis anterior) was also measured, and the effect also occurred after 8 weeks from 30 µg, i.e. parallel to pNfH. Although this was not investigated beforehand either, it may have started earlier.

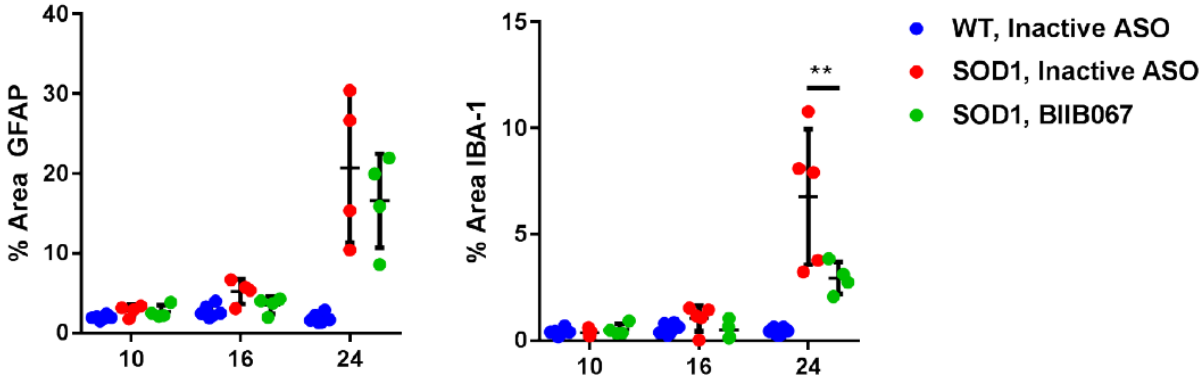
Figure 2: Dose-dependent effect of tofersen on compound muscle action potential (CMAP) recordings in SOD1-G93A mice



Tofersen 30 and 100 µg bolus doses, but not 10 µg, significantly improved compound muscle action potential (CMAP) readings at 8 and 10 weeks of age as compared with the control group ($p<0.0001$). Both the 30 and 100 µg dose levels provided a significant improvement as compared with the 10 µg dose at both 8 ($p<0.05$) and 10 ($p<0.0005$) weeks of age; no significant difference was found between 30 and 100 µg doses at either 8 or 10 weeks of age. The statistical analysis was performed by 2-way analysis of variance (ANOVA) (n=10-12).

It is also interesting that an inflammatory marker (IBA-1) was reduced from 24 weeks. Dose 100 µg at day 50 (5.5 human years) and 100 µg at day 94 (10 human years). From age 24 weeks (18.4 human years, i.e., over 10 years of treatment), the marker was reduced. This is already clearly behind the pNfH effect, which was already changed after a 30-µg dose on Day 56 = 21 days treatment duration = 2.3 human years.

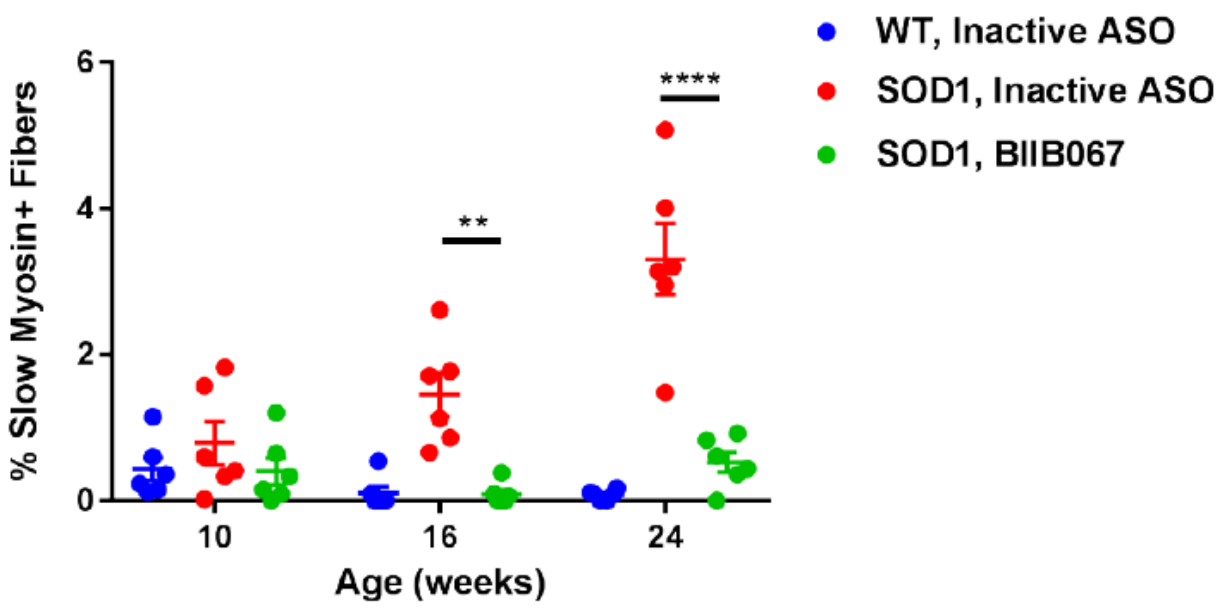
Figure 3: Effect of 2 doses of tofersen on inflammatory markers in the spinal cord of SOD1-G93A mice



GFAP and IBA-1 staining was quantified in the spinal cord of tofersen- or control ASO-treated SOD1-G93A animals and WT animals 10, 16, and 24 weeks of age. While GFAP was not significantly reduced in tofersen-treated SOD1-G93A animals relative to control treated animals ($p>0.05$), IBA-1 was significantly reduced at 24 weeks of age ($p=0.002$; 2-way ANOVA). Ten-week group received one dose of tofersen; 16- and 24-week groups received two doses.

In the same study, muscle composition (reduction in slow myosin-positive fibres) was also investigated. The effect was seen from an age of 16 weeks (12.3 human years, corresponding approximately to 10 years of treatment), but not before.

Figure 4: Effect of 2 doses of tofersen on fiber-type composition of the tibialis anterior muscle in SOD1-G93A mice



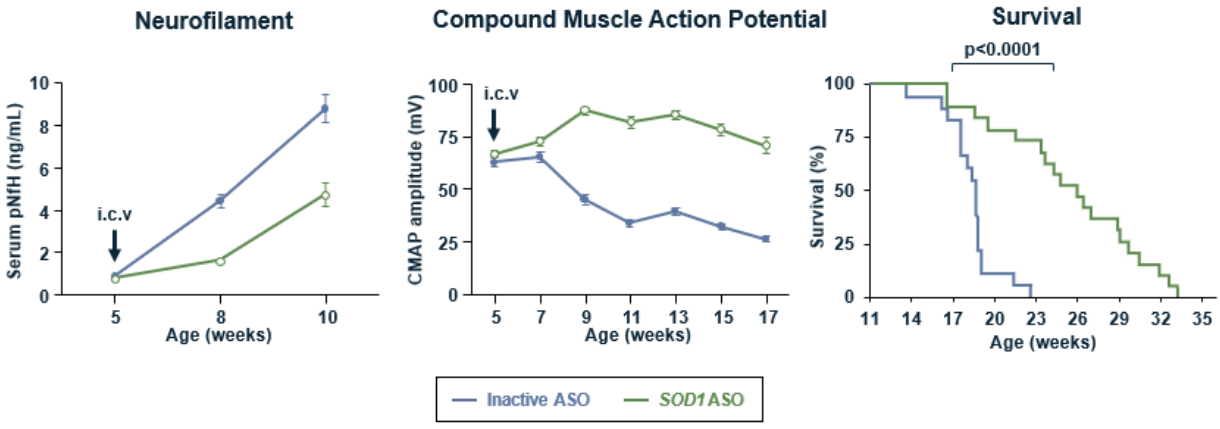
Tibialis anterior muscle from SOD1 animals treated with tofersen had a significantly lower percentage of slow myosin-positive fibers, at 16 weeks of age. Treatment with tofersen significantly decreased the number of slow myosin-positive fibers in the tibialis anterior muscle, both at 16 weeks of age ($p=0.005$) and 24 weeks of age ($p<0.0001$), as compared to muscle of the SOD1, inactive ASO group (2-way ANOVA). Ten-week group received one dose of tofersen; 16- and 24-week groups received two doses.

The biological plausibility as observed by non-clinical data and supported by the effect on the biomarkers suggest a strong mechanistic rationale for the tofersen effect.

Furthermore, non-clinical data in a SOD1 mouse model support this specific pathway: administration of SOD1-lowering ASO → reduced accumulation of the levels of SOD1 protein → reduced levels of neurofilaments = reduced neuronal death → reversed compound muscle action potential loss (after some time lag) → increased survival.

This has been shown in G93A mice in Figure 5.

Figure 5: SOD1-lowering ASO reduces phosphorylated neurofilament heavy chain (pNfH) and prolongs motor performance and survival of SOD1G93A mutant mice



In line with previously performed experiments in mice, the dose-response relationship between tofersen and SOD1 mRNA in rat CNS following IT administration was investigated. The main reason for utilizing the rat in this study was that its larger size facilitates CSF collection. Similar to the mouse, the rat model expresses the full-length human SOD1 gene with G93A mutation, including the 3' UTR which harbours the tofersen binding site, at high levels (Howland, 2002). Tofersen reduced SOD1 mRNA with an EC_{50} of 1.4 µg/g in the lumbar spinal cord and 2.3 µg/g in the cervical spinal cord comparable to the mouse EC_{50} value for the lumbar spinal cord. Both IT and ICV administration of tofersen suppressed SOD1 mRNA and protein in the rat CNS for at least eight weeks following a single administration, consistent with the long half-life of the ASO. SOD1 protein in the CSF was also lowered up to 8 weeks in the SOD1-G93A rat.

In NHPs, SOD1 mRNA was reduced in all brain regions in a dose-dependent manner especially in ALS-relevant regions such as spinal cord and motor cortex while a ~50% reduction in pons and cerebellum was only achieved at the 35 mg high dose. An EC_{50} of 20.7 µ/g tissue was obtained (all brain regions combined). This is a about 10-fold lower potency as compared to transgenic mice or rats expressing human SOD1-G93A. The applicant discussed that this might be in part due to the one base pair mismatch of monkey SOD1 as compared to human SOD1. On the other hand, a very good and comparable knock down of SOD1 mRNA was achieved with tofersen in monkey hepatocyte cell cultures (IC_{50}: 0.98 µM monkey hepatocytes vs. IC_{50}: 1.1 µM human SH-SY5Y cells and IC_{50}: 0.65 µM human A431 cells, respectively). This implicates that the base pair mismatch apparently has no major impact on SOD1 mRNA knock down in monkey cells. One possible explanation could be the formation of neutralizing antidrug antibodies (ADAs) which reduce exposure towards tofersen. In fact, the PK analysis might indicate that there was a tendency towards reduced exposure (area under the concentration time curve (AUC) and maximum observed concentration (C_{max})) and a higher clearance at day 84 as compared to day 1, albeit this appears not to be significant as standard deviations of AUC and C_{max} values were high (refer to Repeat-dose toxicology for details). An ADA analysis has not been performed and thus a firm conclusion on potency differences of tofersen in monkeys and mice/rats expressing humans SOD1 cannot be drawn.

2.5.2.2. Secondary pharmacodynamic studies

There was small but significant increase in IP-10 in response to tofersen treatment (6.9 pg/ml relative to 1.8 pg/ml in untreated cells at 8 µM tofersen) and also other SOD-1 ASOs. The applicant discusses that the degree of change is not expected to translate to a biological effect and is in contrast to the IP-10 induction yielded by treatment with the positive control ASO (513.4 pg/ml). In addition, there was no observed increase in IL-8 in response to any of SOD-1 ASOs tested. It is agreed that the increase is only minor and at tofersen concentrations (8 µM = 57 µg/mL) that are unlikely to be reached in patients (plasma mean C_{max} ~1.4 µg/mL at 100 mg tofersen). Therefore, no tofersen effects on TLR9 and related cytokine production are anticipated in patients.

In total 27 potential off-targets were identified by *in silico* analyses using a transcriptome database. Tofersen was shown to be highly specific for SOD1 mRNA and over 100-fold more potent for the on-target SOD1m RNA than for any of the assessed off-target mRNAs. Overall, no unspecific off-target activities of tofersen are anticipated to occur in patients.

2.5.2.3. Safety pharmacology programme

Safety pharmacology was assessed in an *in vitro* the human Ether-à-go-go-Related Gene (hERG) assay, a single dose toxicity study including assessment of respiratory function and functional observational battery, and during 13-Week and 9-Month Cynomolgus monkey repeat-dose toxicity studies. Tofersen

did not inhibit hERG current up to 34 µM which > 100-fold the plasma concentration reached in patients. In addition, the were no tofersen related cardiovascular effects observed in Cynomolgus monkeys up to the highest dose tested. In the single dose rat study, transient acute tactile hypersensitivity was noted in animals receiving 3 mg. In addition, decreases in arousal, gait, mobility, respiration, and sensorimotor observations were also noted in the 3 mg group corresponding to a human equivalent dose (HED) of 1500 mg. In general, no relevant tofersen-related effects were observed in Cynomolgus monkeys. There were transient neurological signs in single high dose animals with no correlates in clinical and anatomic pathology and were not considered adverse or relevant.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamics (PD) DDI are expected for tofersen; therefore, the lack of respective studies is justified.

2.5.3. Pharmacokinetics

The PK profile of tofersen was characterized in CSF, plasma, and tissues from Cynomolgus monkeys following repeat IT administration (bi-weekly for first month, then monthly) for up to 9 months and in plasma and tissues from mice following single and repeat (bi-weekly for 26 weeks) subcutaneous (SC) administration. Additional studies were conducted to characterize the potential for DDI, including protein binding, metabolite identification, inhibition or induction of CYP450 enzymes, and inhibition or binding to absorptive and efflux transporters.

Considering the nature of tofersen as a second generation, chimeric, mixed backbone ASO and the well know experience with this compound class, the extent of PK studies is regarded as sufficient.

IT is the intended route of administration in humans, and therefore the non-clinical PK studies after IT administration in Cynomolgus monkeys are most relevant for the intended clinical use. Studies after SC administration should be regarded as supportive.

Methods of analysis

The main bioanalytical method, a hybridisation-based ELISA method used for quantification of tofersen in monkey CSF, plasma, and tissues; in the mouse plasma; and rabbit plasma, has been adequately and successfully validated.

Absorption

Following IT injection to Cynomolgus monkeys tofersen concentrations in the CSF declined in a multiphasic manner, with a rapid distribution phase (which dominated the clearance from the CSF), followed by a slower and longer elimination phase. The rapid distribution phase is due to the rapid and broad distribution from the CSF to the CNS tissues with additional clearance due to transfer to the systemic circulation. The estimated CSF elimination half-life ($t_{½}$) was 20 to 45 days, which was similar to the $t_{½}$ in the CNS tissues (31 to 40 days), suggesting equilibrium between CSF and CNS tissues. The mean tofersen predose CSF concentrations generally appeared to increase during the initial bi-monthly administration likely due to accumulation in the CNS tissues and remained at the same level or increased only slightly at higher doses following monthly administration, suggesting that steady state level has been achieved.

Plasma PK demonstrated a dose-dependent peak (C_{max}) and total exposure (area under the concentration time curve from time 0 to 48 hours (AUC_{0-48h})) to tofersen. Plasma exposure increased in a greater than dose-proportional manner with a median time of maximal concentration (t_{max}) between 1 and 4 hours. Plasma concentrations exhibited multiphasic disposition showing a rapid decline during the first 48 hours

post-dose (distribution phase) with a rapid distribution phase followed by a much slower elimination (post-distribution) phase. The post-distribution plasma $t_{½}$ values in the 12 mg/kg dose-group at day 28 and at the 35 mg/kg dose group at day 84 were 22.4 and 50.6 days, respectively. Plasma exposures after repeated administration were generally similar to day 1, with only a slight trend to lower exposure after repeated exposure.

When comparing the corresponding mean post-distribution CSF and plasma concentrations, the mean post-distribution CSF values were generally higher than the corresponding plasma values.

The plasma PK in mice following SC-administration of tofersen was generally similar. Tofersen was absorbed rapidly into the systemic circulation with a t_{max} of 0.5-1 hour. After achieving C_{max}, the plasma concentrations rapidly declined over the next 24 to 48 hours following administration, which along with the multiphasic concentration-time profile suggests rapid and extensive distribution of the drug to tissues. Following repeat SC injection of tofersen once every 2 weeks for 26 weeks a slight increase in exposure (accumulation) to tofersen was observed based on area under the concentration time curve from time 0 to 24 hours ($AUC_{0\text{-}24h}$).

Review of the tofersen plasma concentrations and resulting toxicokinetic (TK) parameters revealed similar systemic exposure between sexes in Cynomolgus monkey and mice.

Distribution

The dose-dependent tissue distribution of tofersen was seen in all the species studied (i.e., monkey, mouse, and rat) with no clear or consistent evidence of a sex difference in distribution to tissues. Following SC administration in mice, tofersen was rapidly and extensively distributed from plasma to the liver and kidney, with no distribution in the brain.

Following IT administration in monkeys, tofersen is broadly distributed to the CNS tissues, with the CNS tissue concentrations at least 90-fold higher than the CSF concentrations. The highest concentrations were observed in the lumbar spinal cord consistent with the route of administration. Relatively high concentrations were observed in the liver and kidney, indicating a significant proportion of the administered dose was distributed from the CSF to the systemic circulation. Tofersen appeared to be cleared very slowly from the CNS tissues. The estimated $t_{½}$ values for tofersen in the CNS tissues ranged from 30.8 to 39.9 days. The estimated $t_{½}$ values for the kidney cortex and liver tissues were 22.5 and 19.8 days, respectively, in the 12 mg/kg dose group and 18.4 and 15.3 days, respectively, in the 35 mg/kg dose group.

Similar to monkeys, tofersen is broadly distributed to the CNS tissues in rats following IT administration.

The investigation of distribution into peripheral organs and tissues was limited to the liver and kidney. There is experience with other oligonucleotides that the liver and kidney are the major organs of distributions after systemic administration (Geary, 2003; Yu, 2007). Furthermore, no further target organs for toxicity have been identified in repeat-dose toxicity studies. Therefore, the lack of extensive tissue distribution studies is acceptable for tofersen as an ASO compound.

In vitro protein binding studies in mice, monkeys and humans showed that tofersen is highly bound to plasma proteins (≥ 95% bound) at the clinically relevant or higher plasma concentrations (0.1 to 30 µg/mL).

Distribution in the blood cells has not been evaluated for tofersen.

Distribution studies in lactating mice on postnatal day 21 following SC administration showed that tofersen appeared in the liver of the F0 mice and is excreted into mouse milk. However, no measurable level was detected in mouse pup liver and brain.

Metabolism

The metabolism of tofersen was investigated in Cynomolgus monkey liver and kidney cortex, after IT lumbar bolus injection (10 mg) of tofersen. All observed metabolites were chain-shortened oligonucleotides with a relative amount of <6% and < 2% of the total peak area in liver and kidney cortex, respectively. Intact tofersen was the most abundant species and accounted for 80 and 75% of the total drug-related peak area in the liver tissue samples, respectively, and 92% of the total drug-related peak area in kidney cortex tissue. Tofersen was also the most abundant species in mice liver and kidney following SC administration. The metabolites of tofersen observed in the kidney and liver of monkeys, suggest that tofersen is metabolized slowly and predominantly via exonuclease (3'- and 5')-mediated hydrolysis. The results of the metabolism studies are consistent with the previous experience with similar classes of ASOs (e.g., 2'-MOE modified ASOs) (Geary, 2003; Yu, 2007). No cross-species comparative metabolism studies, including human, have been performed with tofersen. However, it is expected that the same fate of metabolism also occurs in humans.

Excretion

Non-clinical evaluation of the urinary excretion of tofersen has not been performed but is expected to be minimal as for other 5-10-5 MOE gapmer ASOs following SC drug administration (Geary, 2003). The metabolism of similar classes of ASOs in tissues has been reported to be slow, followed by urinary excretion of the metabolites together with the parent drug as a minor component (Geary, 2003). A similar disposition is expected for tofersen. Given the experience with ASOs, the lack of urinary excretion studies for tofersen is acceptable.

Pharmacokinetic drug interactions

In vitro studies PK DDI studies indicate that tofersen is neither an inducer of CYP450-mediated oxidative metabolism nor a substrate or inhibitor for absorptive or efflux transporters. Therefore, the likelihood of PK DDI interactions is considered low.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

The single dose study in the rat was conducted to assess the effects of local delivery using the clinical route of administration (IT lumbar) in a rodent species.

Decreases in arousal, gait, mobility, respiration, and sensorimotor observations were noted 3 hours post dose in the 3 mg (1500 mg HED) group. No clinical observations were noted on subsequent days, suggesting a transient effect. The no-observed-adverse-effect-level (NOAEL) was determined to be 1 mg.

No ASO specifically targeting rat and mouse SOD1 was used in toxicology studies, due to the lack of sequence homology of tofersen in rodents.

2.5.4.2. Repeat dose toxicity

In a 26-week repeated dose study in CD-1 mice with a SC administration, tofersen was well tolerated. The NOAEL was determined to be the highest dose of 150 mg/kg based on the absence of any adverse findings.

In the 13-week monkey toxicology study, there was transient reduced movement in 2 female monkeys in the highest dose group (35 mg). Therefore, the NOAEL is considered to be at 12 mg/kg.

In the 9-month study, tofersen-related and relevant neurological findings and CSF inflammation were observed in animals administered 35 mg. As a diazepam treatment was considered required for the female administered 35 mg, the neurological findings were considered adverse.

Dose levels of 4 and 12 mg tofersen were well tolerated over a period of 9 months of repeated dosing. In conclusion, the NOAEL upon repeated IT dosing over 9 months is considered 12 mg tofersen.

The neuronal vacuoles – macrovesicular and microvesicular - were not associated with morphological evidence of necrosis.

The microscopic findings in repeat-dose studies in the monkey could be consistent with oligonucleotide uptake and/or cellular activation and cytokine production due to pro-inflammatory effects. The consequences of such uptake and of the potential pro-inflammatory effects were discussed by the applicant, to support the reduced risk of long-term adverse effects.

However, the lack of functional or clinical consequence in relation to microscopic findings such as these, and the long-term consequences of the persistence of tofersen in neurons in the brain is not known.

The calculated safety margin for IT doses in monkeys to IT doses in humans is based on the different CSF volumes in the two species (approximately 10-fold greater absolute CSF volume in humans 2 years or older).

The NOAEL from the 9-month NHP toxicology study was determined to be 12 mg, which converts to a HED of 120 mg (based on the monkey-to-human CSF volume scaling) thus providing safety margins of 1.2-fold, based on CSF volumetric scaling (10x), relative to the human dose of 100 mg.

The development of anti-tofersen antibodies was not investigated in the chronic toxicity studies in rat, mice and monkeys. But ADAs against tofersen were investigated in patients with post-baseline plasma samples for ADAs. Overall, 58.4% of the patients developed treatment-emergent ADAs, but it is difficult to draw conclusions regarding the effects of ADAs on efficacy.

2.5.4.3. Genotoxicity

In vitro, tofersen was negative for an increase in reverse mutations in Ames tests in bacteria and for chromosomal aberrations, polyploidy and endoreduplication in a mammalian chromosome aberration assay in Chinese Hamster Lung (CHL) cells, respectively.

Tofersen did also not increase the formation of micronuclei in polychromatic erythrocytes of mouse bone marrow *in vivo*. A sufficiently high safety margin can be assumed based on toxicokinetic data from repeat-dose toxicity studies in mice. Thus, tofersen exerts no genotoxic potential.

2.5.4.4. Carcinogenicity

As already observed for other phosphorothioate oligonucleotides tofersen did not exert any genotoxic potential *in vitro* and *in vivo*. Its sequence does not contain the prerequisite of ≥10 non-interrupted purine or pyrimidine nucleotide stretches and therefore bares only a negligible potential for triplex formation with genomic DNA.

In NHP, the only PD active species, tofersen did not show any pre-neoplastic or neoplastic lesions and neither immunosuppressive effects nor an endocrine dysfunction were observed in chronic toxicity studies.

In agreement with the CHMP (EMA/CHMP/SAWP/229464/2019) carcinogenicity studies with tofersen are regarded dispensable with respect to the life-threatening nature of ALS, the negative genotoxic potential and the absence of (pre)neoplastic changes, cell proliferation, and immune or endocrine dysfunction in chronic toxicity studies in mice and NHP's.

The carcinogenic potential of tofersen is considered to be low.

2.5.4.5. Reproductive and developmental toxicity

The reproductive and developmental toxicity of tofersen was investigated in a combined study on fertility and embryo-foetal development in mice, a study on embryo-foetal development in rabbits, and on pre-postnatal development in mice. Toxicokinetic data on exposure as well as tofersen tissue concentrations were obtained within some of these studies. In all studies, tofersen was administered via SC injection once every other day to ensure maximum exposure of adult tissues (and especially reproductive organs) and fetuses to tofersen.

In the pivotal combined study on fertility and embryo-foetal development in mice, tofersen was not found to have any adverse effects on either male or female fertility at any dose (3, 10, or 30 mg/kg). However, in male mice, evaluation revealed that tofersen-related histopathologic findings in testes and epididymides were detected at ≥ 10 mg/kg. Similar microscopic findings are known class effect of ASOs. Findings such as tofersen-related vacuolated macrophages/cytoplasmic vacuolation/macrovesicular vacuolation were also found in other organs (please refer to repeat-dose toxicity). The evaluation also revealed that the prostate in the 30 mg/kg dose group had a significant increase in absolute and ratio of organ weight to body weight. The NOAEL for male reproductive and general toxicity of tofersen was 10 mg/kg.
In female mice, no tofersen-related adverse effects were noted. There were no tofersen-related effects on any fetal external, visceral, or skeletal malformations or variations. The maternal general toxicity and reproductive/developmental NOAEL for tofersen was 30 mg/kg, the highest dose tested (> 50 times the human exposure [AUC] following 100 mg tofersen).

In the study on embryo-foetal development in rabbits, tofersen did not result in any mortality. No tofersen-related adverse clinical signs or tofersen-related findings were present in maternal rabbits. There were no tofersen-related effects on embryo-foetal survival or foetal body weights and no tofersen-related fetal external, visceral, or skeletal malformations or variations. The maternal and developmental NOAEL for tofersen was 30 mg/kg, the highest dose tested (more than 40-times the human exposure following 100 mg/month tofersen).

In a pre-postnatal development study in mice, tofersen administration did not produce tofersen-related mortality in F0 generation females or F1 generations pups at any dose (3, 10 or 30 mg/kg). There were no tofersen-related effects on either the F0 females or the F1 generations (males and females). The NOAEL for both the F0 and F1 generations was 30 mg/kg, the highest dose tested. Plasma exposures (area under the concentration time curve from time 0 to the last measured concentration ($AUC_{0\text{-last}}$)) collected in pregnant mice in the pivotal fertility and embryo-fetal development (FEFD) Study indicate that a dose of 30 mg/kg in mice is >50 times the human exposure [AUC] following 100 mg of tofersen.

Placental transfer of tofersen was not investigated. Overall, maternal exposure does not appear to lead to any toxicologically relevant exposure in the developing foetus, indicating that tofersen may not cross the placenta, or may do so only to a very limited extent.
Tofersen was detected in the milk samples of F0 maternal mice from all tofersen-dosed animals following dose administration on LD 13. No clear dose-dependent increase in tofersen concentration in milk was observed. However, tofersen was also detected in three out of the six control samples analysed. Why tofersen was detected in the control samples, however, could not finally be clarified. Given these

limitations, these findings should only be considered as qualitative detection of tofersen in mice milk samples.
F0 generation mice administered tofersen had measurable levels of tofersen in the liver on lactation day (LD) 21; however, tofersen was not detected in liver and brain tissue from the F1 generation pups (on postnatal day 21), indicating that it may be degraded in the gastrointestinal tract of nursing F1 pups.

As tofersen is not pharmacologically active in mice and rabbits, the results of the developmental and reproductive toxicity (DART) studies would primarily reflect only the evaluation of the toxicity of its chemical structure. While the available data on DART indicate that the backbone of the ASO does not result in toxicity, adverse events related to SOD1 down-regulation are currently unknown and therefore are reflected in sections 4.6 and 5.3 of the SmPC.

2.5.4.6. Toxicokinetic data

Drug concentration data for CSF (monkey only), plasma (monkey and mouse only), and evaluated tissues (monkey, mouse, and rat) were assessed to characterize the PK properties of tofersen. Following bolus IT administration in monkeys, tofersen concentrations in CSF declined in a multiphasic manner, with a rapid distribution, followed by a slower and longer elimination phase. Following SC administration in mice, tofersen was rapidly absorbed into the systemic circulation, then similar to monkeys, declined over the next 48 hours due to extensive distribution to systemic tissues. Distribution of tofersen was dose dependent in all species studied, with broad distribution to CNS tissues following IT administration.

TK plasma exposure was determined following administration of tofersen once every other day by SC injection at 3, 10, and 30 mg/kg in pregnant mice and pregnant rabbits in the reproductive toxicity program. Toxicokinetic parameters were evaluated in female mice on gestation day (GDs) 6 and 14 and in female rabbits on GDs 7 and 19.

In pregnant mice, toxicokinetic evaluation indicated that evidence of systemic exposure to tofersen was observed in all tofersen treated pregnant mice following SC injection on GDs 6 and 14. High variability in the plasma concentration values was observed between animals at all dose levels on GDs 6 and 14 with concentrations quantifiable up to 24 hours post-dose. The t_{max} values were observed at 1- or 2-hours post-dose on GDs 6 and 14 and apparent $t_{1/2}$ was observed at 5.91 hours at 10 mg/kg on GD 14. In general, tofersen plasma exposure based on C_{max} and $AUC_{0\text{-last}}$ increased in a dose-proportional manner over the dose range evaluated and this increase in exposure was in general dose levels, except between 10 and 30 mg/kg on GD 6 where the increase was greater than approximately dose proportional (>2-fold) for $AUC_{0\text{-last}}$. A slight accumulation (AUC) of tofersen was observed at 3 and 10 mg/kg/dose on GD 14 relative to GD 6.

In the TK evaluations in pregnant rabbits, the t_{max} was mostly observed at 1 and 2 hours after SC injection of tofersen on DGs 7 and 19. Systemic exposure to tofersen (as assessed by mean C_{max} and $AU_{0\text{-last}}$) increased with increasing in dose levels on DGs 7 and 19. The observed increase in both C_{max} and $AUC_{0\text{-last}}$ was approximately dose proportional over the whole dose range tested except between 3 and 10 mg/kg/dose on DG 7 where the exposure increased in a less than proportional manner for C_{max}. The mean $t_{1/2}$ ranged between 2.44 and 2.90 hours on DGs 7 and 19. Repeated subcutaneous injections of tofersen at 3, 10 and 30 mg/kg/dose once every other day on DGs 7 through 19 did not revealed accumulation as assessed by mean $AUC_{0\text{-last}}$.

2.5.4.7. Local Tolerance

Local tolerance was assessed in repeat-dose toxicity studies. Inflammation and haemorrhage were observed at the injection site in mice but resolved after recovery. In NHPs, IT administration of tofersen

caused mononuclear inflammatory cell infiltrates in the meninges at the injection site of the lumbar spinal cord in males and females at ≥ 4 mg, possibly as a local pro-inflammatory effect caused by RNAse H-based ASOs. Remnants of such inflammatory infiltrates were still detectable after 13 weeks of recovery, although at a lower magnitude.

2.5.4.8. Other toxicity studies

Impurities

Independent of the mixture and impurity composition, the toxicity profiles of the 3- and 9-month toxicity and impurity studies were comparable with no supplementary signs of toxicity and similar TK profiles of the parent drug at nearly the same dose level. Margins at NOAEL were 1 – 3.5 times to the corresponding human dose of 100 mg with regard to the HED concept monkey-to-human CSF volume scaling (human CSF 10 x > NHP CSF) for IT administration.

Therefore, on the basis of the impurity profile of all mixtures and batches tested the qualified level for an individual impurity was the maximum percentage used.

Phototoxicity

Tofersen absorbed light peaking at approximately 260 nm that exhibits a spectral pattern that is typical for nucleic acids and consistent with the known absorbance spectrum of oligonucleotides.

In general, oligonucleotides are exempted from ICH S10 requirements (EMA/CHMP/ICH/752211/2012).

Tofersen did not induce ophthalmological or cutaneous alterations in repeat-dose toxicity studies.

The phototoxic potential for tofersen is considered to be low and therefore, no studies concerning phototoxicity testing were conducted or are deemed necessary.

2.5.5. Ecotoxicity/environmental risk assessment

The applicant submitted an environmental risk assessment (ERA) Phase I for the active substance tofersen.

Based on prevalence data taken from the orphan designation for tofersen (EU/3/16/1732) for the treatment of ALS and the treatment regime, the Fpen has been refined and used for calculation of a refined $PEC_{surfacewater}$resulting in a value of 0.0005 µg/l. As the $PEC_{surfacewater}$ does not exceed the action limit of 0.01 µg/l it is concluded that a Phase II testing is not triggered. The provided calculation is acceptable, and the risk assessment stopped in Phase I of the procedure.

For screening on persistence, bioaccumulation, toxicity (PBT) a study on log K_{ow} according to OECD 107 has been provided. As the resulting log K_{ow} values of -1.11 (pH 5), -1.83 (pH 7) and -1.90 (pH 9) does not exceed the trigger value no further screening for persistence, bioaccumulation and toxicity is deemed necessary.

Table 2 : Summary of ERA main study results

Substance: tofersen			
CAS-number (if available): 2088232-70-4			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	study is missing	-1.11 (pH 5) -1.83 (pH 7) -1.90 (pH 9)	Potential PBT (N)
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.0005	µg/L	> 0.01 threshold (N)
Other concerns (e.g. chemical class)			(N)

The PEC surface water value is below the action limit of 0.01 µg/L and tofersen is not PBT substance as log Kow does not exceed 4.5.

2.5.6. Discussion on non-clinical aspects

The non-clinical pharmacology programme evaluated the selectivity and efficacy of tofersen using *in vitro* and *in vivo* studies. *In vitro*, tofersen showed suppression of SOD1 mRNA in two human cell lines and Cynomolgus primary hepatocytes to a similar extent.

In vivo pharmacological activity of tofersen was demonstrated in transgenic mouse and rat models, each expressing human SOD1 with a G93A mutation, and in Cynomolgus monkeys. The non-clinical findings provide a mechanistic rational for the effect of tofersen and support a delay in the treatment effect of tofersen.

Bioinformatic analysis was performed to identify potential off target transcripts based on the primary sequence of tofersen. The *in silico* predicted off-targets were either not expressed in the CNS, did not show evidence of reduction by tofersen *in vitro*, or had a much lower sensitivity to tofersen compared to on-target SOD1 mRNA inhibition (>100 fold by IC_{50} values) in human cells.

Safety pharmacology assessments for potential CNS, respiratory, and cardiovascular effects were assessed in a single dose rat study and in 13-week and 9-month toxicity studies in Cynomolgus monkeys (NHPs). In rats, decreases in arousal, gait, mobility, respiration, and sensorimotor observations were seen in the 3 mg dose group corresponding to a HED of 1500 mg. In NHPs, there were no relevant tofersen-related effects on any of the safety pharmacology endpoints evaluated. In addition, tofersen demonstrated no relevant potential for cardiac hERG channel inhibition.

PK studies with tofersen were performed in CSF, plasma, and tissues from Cynomolgus monkeys following IT administration, the intended route of administration in humans. In addition, supportive PK studies were conducted in mice following SC administration. Tofersen showed a PK profile typical for ASOs.

Following IT injection to cynomolgus monkeys tofersen concentrations in the CSF declined in a multiphasic manner, with a rapid distribution phase from CSF to CNS tissues followed by a longer elimination phase into the systemic circulation, where also biphasic decline was observed. The $t_{1/2s}$ of tofersen were 20 to 45 days in CSF, 31 to 40 days in CNS tissues and up to 50.6 days in plasma.

Following IT administration in monkeys, tofersen is broadly distributed to the CNS tissues, with the CNS tissue concentrations at least 90-fold higher than the CSF concentrations and relatively high concentrations in the liver and kidney. Following SC administration in mice no distribution in the brain was observed. Tofersen is highly bound to plasma proteins (≥ 95% bound) in mice, monkeys, and human.

Tofersen is metabolized slowly and predominantly via exonuclease (3'- and 5')-mediated hydrolysis to chain-shortened oligonucleotides but intact tofersen was the most abundant species in liver and kidney.

Non-clinical evaluation of the urinary excretion of tofersen has not been performed but is expected to be minimal as for other 5-10-5 MOE gapmer ASOs.

Tofersen is neither an inducer of CYP450-mediated oxidative metabolism nor a substrate or inhibitor for absorptive or efflux transporters and the likelihood of PK DDI is considered low.

Considering the nature of tofersen as a second generation, chimeric, mixed backbone ASO and the well known experience with this compound class, the extent of PK studies with tofersen is regarded as sufficient.

Overall, the toxicology programme revealed that tofersen was well tolerated. The microscopic findings in repeat-dose studies in the monkey could be consistent with oligonucleotide uptake and/or cellular activation and cytokine production due to pro-inflammatory effects. The consequences of such uptake and of the potential pro-inflammatory effects were discussed by the applicant, to support the reduced risk of long-term adverse effects. However, the lack of functional or clinical consequence in relation to microscopic findings such as these, and the long-term consequences of the persistence of tofersen in neurons in the brain is not known. The development of anti-tofersen antibodies was not investigated in the chronic toxicity studies in rat, mice and monkeys. But ADAs against tofersen were investigated in patients with post-baseline plasma samples for ADAs. Overall, 58.4% of the patients developed treatment-emergent ADAs, but it is difficult to draw conclusions regarding the effects of ADAs on efficacy.

Tofersen was not genotoxic, and no carcinogenicity studies have been performed due to the life-threatening nature of ALS, the negative genotoxic potential, and the absence of (pre)neoplastic changes, cell proliferation, and immune or endocrine dysfunction in chronic toxicity studies in NHPs.

The potential DART of tofersen was investigated in mice and rabbits. These studies do not indicate harmful effects with respect to reproductive toxicity at therapeutic doses. Considering that tofersen is not pharmacologically active in mice and rabbits, these studies do not provide data regarding the pharmacological activity of tofersen in terms of its potential effects on reproduction or development. This limits the predictive value of these studies in terms of human safety. In this regard, the applicant points out, that an appropriate rodent surrogate ASO for SOD1 was not identified. It is important to note that the use of surrogates in non-clinical safety studies is challenging in that they may sometimes produce a different pattern of toxicity unrelated to target pharmacology. Findings from studies in SOD1 KO-mice have shown that loss of SOD1 has harmful effects on fertility, pregnancy and development. The interpretation of the clinical relevance of these toxic effects in SOD1 KO-mice could be difficult.
The applicant highlighted that the effects of SOD1 KO in humans by the SOD1 ASO is not 100% and is more reminiscent of SOD1 heterozygous mice. A literature review of the available data on the effects of SOD1 down-regulation indicates that the potential effects of ASO (tofersen) on reproduction and development are likely to be low; however, they are currently unknown. The lack of pharmacologically relevant studies with regard to pregnancy and fertility is now also adequately reflected in sections 4.6 and 5.3 of the SmPC.

Tofersen $PEC_{surfacewater}$ value is below the action limit of 0.01 μg/L. and is not a PBT substance as log K_{ow} does not exceed 4.5.

Assessment of paediatric data on non-clinical aspects

A product specific PIP waiver was granted (EMEA-002403-PIP01-18). It is widely accepted that SOD1-ALS only occurs in the adult population. The European Medicines Agency has waived the obligation to submit the results of studies with tofersen in all subsets of the paediatric population in ALS. It is endorsed that no juvenile toxicity studies have been performed.

2.5.7. Conclusion on the non-clinical aspects

Overall, the primary pharmacodynamic studies provided adequate evidence that tofersen shows good efficacy *in vitro* and in relevant animal models. No relevant off target effects are anticipated based on secondary PD studies. Safety pharmacology studies did not reveal safety signals for patients.

From the *PK* point of view, the monkey was the most relevant species for non-clinical efficacy and safety studies.

Overall, the toxicology programme revealed that tofersen was well tolerated.

The wording of sections 4.6 and 5.3 of the SmPC in relation to the lack of pharmacologically relevant DART studies has now been revised to adequately reflect this aspect.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The tofersen clinical development program (CDP) consists of two completed and two ongoing clinical studies: Study 233AS101 Parts A, B, and C (complete) and open-label extension Study 233AS102 (ongoing) in participants with SOD1-ALS and Study 233HV101 (complete) in healthy volunteers and Study 233AS303 or ATLAS (ongoing) in presymptomatic SOD1 mutation carriers. No efficacy data from Study 233AS303 have been included in this application because the study remains blinded.

Separate to the CDP, there is an ongoing Expanded Access Program (EAP) (section 2.6.5.7.1).

The applicant has also in place an ongoing evidence generation plan (section 2.6.5.7.1). Continual long-term data generation in this rare, highly heterogeneous disease is important and could probably help further contextualize the longer-term benefit/risk of tofersen and better understand its long-term impact on disease progression and survival.

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

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

- **Tabular overview of clinical studies**

Table 3: Listing of clinical studies

Study Identifier [Countries]	Study Design	Test Products, Dose Regimen, Route of Administration	Primary Objectives	Number of Partici-pants Enrolled	Duration of Treatment	Study Status
Studies in Participants with SOD1 ALS (weakness attributable to ALS and a confirmed SOD1 mutation)						
233AS101 CSR 233AS101 Parts A and B CSR 233AS101 Part C [Australia, Belgium, Canada, Denmark, France, Germany, Italy, Japan, Republic of Korea, Poland, United Kingdom, United States]	**Part A** Phase 1/2 randomized, double-blind, placebo-controlled SAD study in participants with ALS[a]	Placebo vs. tofersen 10, 20, 40, 60 mg Intrathecal bolus injection	Safety, tolerability, and PK.	Tofersen: 15 Placebo: 5	Single dose	**Complete**
	Part B Phase 1/2 randomized, double-blind, placebo-controlled MAD study in participants with SOD-1 ALS	Placebo vs. tofersen 20, 40, 60, 100 mg Intrathecal bolus injection	Safety, tolerability, and PK.	Tofersen: 38 Placebo: 12	12 weeks	**Complete**
	Part C Phase 3 randomized, double-blind, placebo-controlled study in participants with SOD1-ALS	Placebo vs. tofersen 100 mg Intrathecal bolus injection Dosage regimen: 3 loading doses administered 2 weeks apart followed by maintenance doses every 4 weeks thereafter Treatment duration: 24 weeks (8 doses)	Efficacy Primary endpoint: Change from baseline to Week 28 in the ALSFRS-R total score	Tofersen: 72 Placebo: 36	24 weeks	**Complete**
233AS102 CSR 233AS102 Interim 1 CSR 233AS102 Interim 2 [Belgium, Canada, Denmark, France Germany, Israel, Italy, Japan, New Zealand, United Kingdom, United States]	Phase 3 open-label extension for participants who completed Study 233AS101	Tofersen 100 mg[b] Intrathecal bolus injection Dosage regimen: 3 loading doses administered 2 weeks apart* followed by maintenance doses every 4 weeks thereafter. *Participants who received tofersen in Study 101 Part C receive 2 loading doses of tofersen 100 mg on Days 1 and 29, and placebo on Day 15 to maintain the study blind. Treatment duration: At least 152 weeks and up to 360 weeks	Long-term safety and tolerability Primary endpoint: Incidence of AEs and SAEs	Tofersen: 139 Participants with SOD1-ALS who completed Study 101	Up to 360 weeks	Ongoing

Study Identifier [Countries]	Study Design	Test Products, Dose Regimen, Route of Administration	Primary Objectives	Number of Partici-pants Enrolled	Duration of Treatment	Study Status
Study in Healthy Volunteers						
233HV101 CSR 233HV101 [United States]	Phase 1 open-label study of a single-dose of radiolabelled tofersen (99mTc-MAG3-BIIB067) coadministered with unlabeled tofersen in healthy volunteers	Tofersen 40 mg and 100 mg containing up to 111 to 148 MBq 99mTc-MAG3-tofersen Intrathecal bolus injection	Evaluate CNS distribution of radiolabelled tofersen given with unlabelled tofersen	8[c]	Single dose	**Completed**
Study in Pre-symptomatic SOD1Mutation Carriers						
233AS303 (ATLAS) [Australia, Belgium, Brazil, Canada, France, Germany, Italy, Japan, Republic of Korea, Poland, Spain, Sweden, United Kingdom, United States]	Phase 3 randomized, placebo-controlled study with a longitudinal natural history run-in and open-label extension	Placebo vs. tofersen 100 mg Intrathecal bolus injection	Efficacy	150 (planned)	2 years	Ongoing[d]

MAD = multiple ascending dose; MBq = megabecquerel; PD = pharmacodynamics; PK = pharmacokinetics; SAD = single ascending dose

a A total of 6 participants in Study 233AS101 Part A did not have a SOD1 mutation, since this was not required prior to Protocol Version 2.0. None of these 6 participants enrolled in Study 233AS102; 2 only received placebo while 4 received a single dose of tofersen.

b A blinded loading dose period was incorporated in Study 233AS102 for participants who enrolled after completing Part C of Study 233AS101; blinded placebo or tofersen was administered at Day 15 for those who received tofersen or placebo in Study 233AS101 Part C respectively

c 5 of the 8 participants were enrolled at a site that was ultimately discontinued due to Good Clinical Practice noncompliance; data from these participants were reported but not analysed.

d Study 233AS303 will enrol n=150 participants in the observational run-in period (Part A) of the study, of which ~34 participants (~28 in Part B/C and ~6 in Part D) will enter the interventional study period. No data were available from Study 233AS303 as of the data cutoff for this application.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Analytical Methods

In total, 12 different bioanalytical assay were reported. The PK of tofersen was analysed in plasma, serum, CSF, and CNS tissue via hybridisation enzyme-linked immunosorbent assay (ELISA). SOD1 PD was analysed in CSF (sandwich immunoassay and Liquid chromatography–mass spectrometry) and CNS tissue (sandwich immunoassay). The biomarkers pNfH and neurofilament light chain (NfL) were both

each analysed in CSF (immunoassay and single-molecule immunoassay, respectively) and plasma (immunoassay, single-molecule immunoassay, and sandwich immunoassay), and immunogenicity (anti-tofersen antibodies) were analysed in plasma (immunoassay). The lower limit of quantification in CSF and plasma were 1 ng/mL.

Pharmacokinetic data analysis

The PK of single and multiple doses of IT administered tofersen was characterised in plasma and CFS. CSF and Plasma PK were analysed using non-compartmental analysis (NCA) in studies 233AS101 and 233AS102. In addition, PK data from studies 233AS101 and 233AS102 were analysed using population PK (popPK) modelling and simulation techniques using the nonlinear-mixed effects modelling approach with NONMEM software.

Evaluation and Qualification of Models

Standard model evaluation and validation methods such as goodness-of fit plots, visual predictive checks, and precision of parameter estimates were used.

Statistical methods

For NCA PK results $AUC_{0\text{-}24h}$, C_{max} and t_{max} results were presented with standard descriptive statistics.

Absorption

IT injection of tofersen into the CSF allows the drug to be administered in close proximity to the site of action within the CNS. Following IT bolus administration, tofersen was rapidly transferred from CSF into the CNS tissue and systemic circulation, with a median t_{max} of 2 to 6 hours in plasma.

In CSF steady state after multiple dosing was achieved immediately after the loading dose period (3 doses administered every 2 weeks). The maximum CSF trough concentration occurred at the third dose, which was the last dose of the loading dose period. There was little to no accumulation with monthly dosing after the loading dose period; the accumulation ratio appears to be less than 2-fold. This CSF accumulation indicates that the effective $t_{1/2}$ of tofersen in CSF is approximately 1 month. Trough tofersen CSF concentrations stabilized at steady state and remained generally unchanged for the remainder of the administration period.

In plasma dose-dependent mean plasma C_{max} was observed within a few hours after dosing, indicating that the drug was rapidly transferred from the site of administration (CSF) into systemic circulation. Mean plasma drug concentrations typically declined rapidly after reaching C_{max}, mainly due to extensive distribution from plasma to systemic tissues. After single dose plasma concentrations were below limit of quantification at Day 8 (168 hours post dose) as well as later timepoints, precluding estimation of the terminal plasma $t_{1/2}$. After multiple dose administration the AUC and C_{max} values were generally consistent between the first and last doses.

The trough plasma concentration versus time profiles appear to stabilize in the 2 months after the loading dose period (until Day 85), after which a slow drift toward higher trough concentrations over time is observed. The reason for this drift is not understood at this time.

Distribution

Tofersen is distributed extensively in CNS tissues with highest concentrations present close to the site of administration (spinal cord tissues), as confirmed by the analysis of autopsy tissues from three tofersen-treated patients in Study 233AS102. After reaching the peak level, plasma concentrations of tofersen declined rapidly, likely due to extensive distribution to systemic tissues.

Elimination

Plasma elimination $t_{1/2}$ could not be estimated, as plasma concentrations of treated participants were below the limit of quantification at 8 days (168 hours postdose) and after. The observed descending portions of the curves probably represent redistribution phases. The effective $t_{1/2}$ of tofersen in cerebrospinal fluid was approximately 1 month.

The route of excretion was not specifically investigated in humans. The expected primary route of elimination is likely via urinary excretion of nucleotides after tofersen is metabolized slowly by exonuclease-mediated hydrolysis.

The metabolism of tofersen has been evaluated in CD-1 mice and in cynomolgus monkey. The results in liver and kidney samples are consistent with the previous experience with similar classes of ASOs (e.g., 2'-MOE modified ASOs). In addition to the parent compound, putative oligonucleotide metabolites, consistent with nuclease-mediated metabolism, were evident in the kidney and liver samples. Based on the previous experience with similar classes of ASOs, major circulating or unexpected metabolites for tofersen would not be expected in humans.

Dose proportionality and time dependencies

In CSF tofersen exposures (troughs) increased with dose level; the increase was less than dose proportional from 20 to 100 mg. The tofersen CSF concentration in participants receiving tofersen 10 mg was below the limit of quantitation at all sampling timepoints. Concentrations were highest in the 100 mg group, lowest in the 20 mg group, and similar in the 40 and 60 mg groups. Concentrations of the 100 mg dose were approximately 2 to 3 times higher than those observed for the 20 mg dose.

In plasma significant tofersen plasma PK variability was observed, and formal PK linearity analysis was not performed due to the small sample size; however, in plasma it seems that exposure tend to increase more than dose-proportional from 20 mg single dose and multiple dose onwards.

Population PK modelling

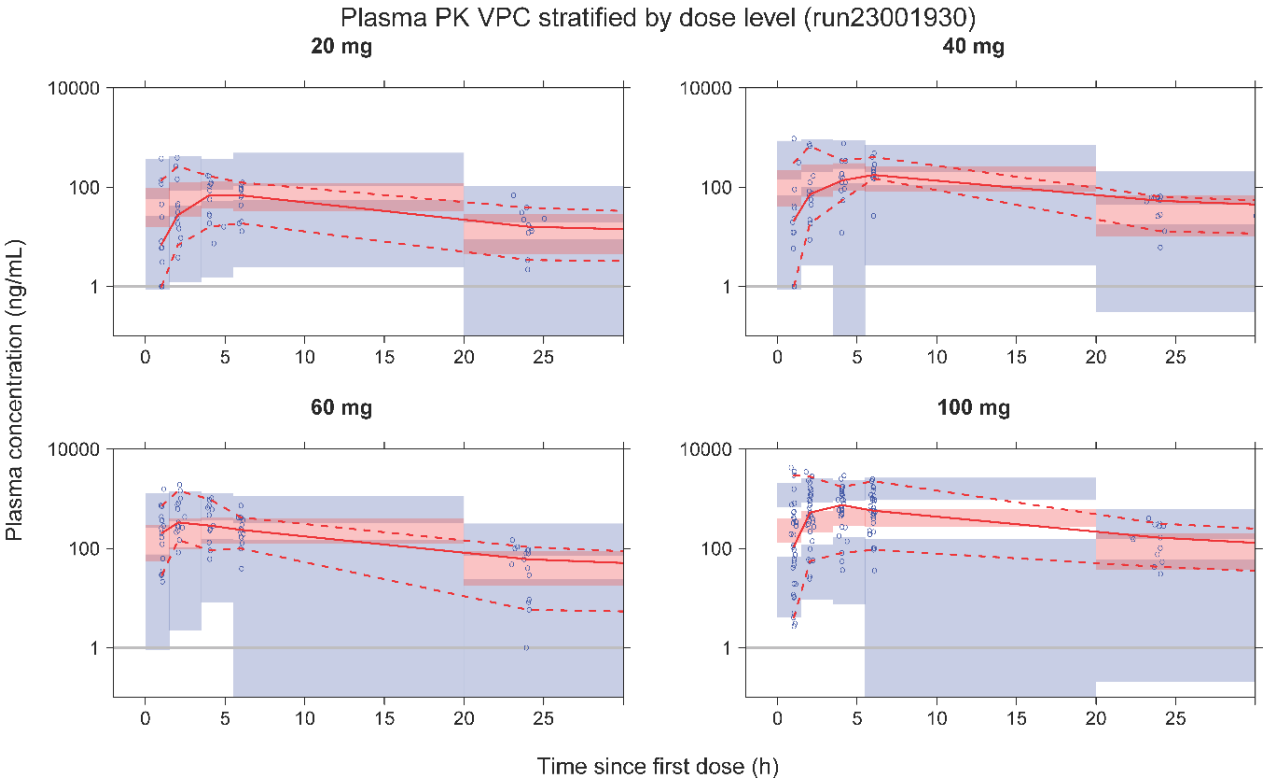
PK data from studies 233AS101 and 233AS102 were analysed using popPK modelling and simulation techniques. Two different models for plasma and CSF PK were developed. The plasma PK data were best described by a two-compartment model with depot compartment and first order elimination. The absorption rate from CSF to plasma (K_A) of the final covariate model was 0.142 L/hr, the plasma clearance (CL) was 9.04 L/hr and central volume of distribution (V2 or $V_{central}$l) was 58.2 L. The inter-individual variability (IIV) were moderate to high on CL (IIV = 69.6 %CV), K_A (IIV = 49.8 %CV), $V_{central}$ (IIV = 129 %CV), $V_{peripheral}$ (IIV = 131 %CV) and inter-compartmental clearance (IIV = 66.8 %CV). The following covariates were identified: ADA on CL (positive response of ADA antibodies decreased the plasma clearance of tofersen), body surface area (BSA) baseline level on CL, and sex on $V_{central}$. Relative standard errors were below 25 %.

The CSF PK data was best described by a one-compartment model. The elimination rate (K) was 0.000876 /h (IIV = 84.1 %CV) and volume of distribution V was 8730 L (IIV = 105 %CV) a proportional residual unexplained variability at 79% was estimated. The CSF PK model was used to describe the PKPD relationship with SOD1 CSF and NfL plasma measures. Simulations for different doses higher than the proposed 100 mg IT dose were carried out (please refer to section Pharmacodynamics). Admittedly, the CSF PK model has limitations due to the data on which it is based on. However, it is regarded difficult to get more/better conclusions from the data by refining the model.

Using pharmacometric software, VPCs stratified by nominal dose level for the plasma PK model were generated, which were not identified in the popPK report provided by the applicant (see the Figure 6 below; x-axis is limited to the first day only and shows only data following the first dose in each individual). The model appears to give an overall acceptable description of the plasma PK data during the first 24 hours, however, observations during the absorption phase are slightly over-predicted.

Upon further scrutiny of the raw data for plasma PK, it was found that ~50% of the observations have the concentration set to the value 1.0 ng/mL (same as the lower limit of quantification) and these values were included in the final PopPK model, treating them as a continuous variable. Handling of the data using M3 was submitted on request. The run with M3 method shows an extremely high condition number and high RSE on all THETAs, suggesting that the model is highly unstable and not able to show much advantage compared to the original based model in fitting the data.

Figure 6: Visual predictive check (VPC) of plasma drug concentrations versus time since first dose for the tofersen population pharmacokinetic model.



Data are presented on a semi-log scale. Observed data (open circles) are presented as median (red solid line), 5th and 95th percentiles (red dashed lines). The predictions are based on 500 simulated datasets. For simulations, the 95% confidence intervals of the corresponding median, 5th and 95th percentiles are shown as shaded areas. The lower limit of quantification (1 ng/mL) are shown as a horizontal grey line. Only observations following each subjects' first dose are included.

Special populations

Impaired renal function

The PK of tofersen has not be investigated in a dedicated renal impairment study.

Impaired hepatic function

The PK of tofersen has not be investigated in a dedicated hepatic impairment study.

Gender

Overall, 49 and 48 % of the PK population from studies 233AS101 and 233AS102, respectively, were male and 51 and 52 % female.

Based on popPK modelling, sex was identified as statistically significant covariate (factor -0.579) on $V_{central}$ for tofersen in plasma, leading to a lower $V_{central}$ for female patients of about -57.9 %. Thus, $V_{central}$ in females for tofersen in plasma is expected to be about 24 L compared to males who are expected to have a $V_{central}$ of about 52 L.

Body weight

Baseline body weight was not a significant covariate for the PK parameters such plasma volume, and CL. However, BSA, an alternative measure of body size, was identified as a covariate of CL of tofersen.

Race

Among the PK study population in study 233AS101 and 233AS102, the majority was white (n=111 [63 %] and n=83 [59 %]). Overall, 51 (29 %) and 44 (31 %) participants were of unknown race, 10 (6 %) and 9 (6 %) Asian, and each 4 (2 % and 3%, respectively) where of "Other" races.

Race was not identified as a statistically significant covariate affecting the PK of tofersen. Observed PK difference across different races are likely attributed to differences in BSA. No dose adjustments based on race are proposed by the applicant.

Body weight and Body surface area

Among the study population, body weight ranged from 42.5 to 146.5 kg (mean=78.7 kg) and 42.5 to 146.5 kg (mean=83 kg) in study 233AS101 and 233AS102, respectively. BSA ranged from 1.37 to 2.71 m^2 (mean=1.92 m^2) and 1.38 to 2.71 m^2 (mean=1.95 m^2), respectively.

Based on popPK modelling BSA was identified as statistically significant covariate (factor 1.26) on CL for tofersen in plasma, leading to a varying CL between -35 to +52 % (i.e. CL=5.8 L/h and 13.7 L/h, respectively for BSA values of min=1.37 and max=2.71) compared to the typical value of 9.05 L/h (BSA=1.94).

Elderly

Among the study population, age ranged from 21 to 78 years (mean=49 years) and 23 to 75 years (mean=49 years) in study 233AS101 and 233AS102, respectively. Age was not identified as significant covariate in any of the PK analyses seems not to have an impact on the plasma PK of tofersen.

Study 233AS101 Part B: Participant were ages from 21 to 66 years (mean = 51.3 years), with the placebo group having a slightly higher mean participant age (58.4 years) than the active treatment groups (45.0 to 55.3 years). In total, three participants (15 %) were aged ≥ 65 years.

Study 233AS101 Part B: Participant were aged from 26 to 75 years (mean=48.5 years), with the 40 mg treatment group having a slightly higher mean participant age (58 years) than the other groups (41.5 to 49.2 years). In total, five participants were aged ≥ 65 years.

Study 233AS101 Part C: The age ranged from 23 to 78 years (mean=49.2 years), with an overall higher mean participant age in the placebo group (51.2 years) compared with the tofersen group (48.1 years), this difference was driven by a difference in the "enriched" subgroup, where the mean age was higher in the placebo group (54.0 years) compared with the tofersen group (47.3 years). In total, 14 participants (13 %) were aged ≥ 65 years.

Study 233AS102: n=13 age 18 – 35 years, n = 57 age 35 - < 50 years, n = 52 age 50 - < 65 years, n = 17 ≥ 65 years (overall n=139)

Based on popPK modelling age was not identified as a covariate affecting PK. No dose adjustment based on age is proposed by the applicant.

Children

Tofersen is not planned to be used in paediatrics below the age of 18 years. The PK of tofersen has not been studied in paediatrics. In 2018, a waiver was granted for paediatrics from birth to less than 18 years of age (EMEA-002403-PIP01-18 and EMA/670855/2018).

Immunogenicity

Among the PK study populations, 33 (19 %) and 22 (16 %) patients were ADA positive in study 233AS101 and 233AS102, respectively.

Based on a preliminary popPK analysis, ADA were identified as statistically significant factor influencing the clearance of tofersen in plasma, leading to a lower tofersen clearance of about 5.16 L/h in ADA positive patients compared to ADA negative patients.

The popPK model was updated using data from the July 2021 data cut for Study 233AS102 and refined resulting in a model with time varying ADA effect on CL. ADA positive patients are expected to have a 32.2% reduced clearance of tofersen (compared to 42,9% in the previous model) leading to higher Ctrough concentrations for ADA positive patients (9.33 ng/mL vs 3.64 ng/mL). The applicant provided no mechanistic interpretation of this phenomenon. The applicant plans to further evaluate the impact of ADAs on efficacy, on the incidence of serious neurological events and other areas of interest, which may arise, in the ongoing clinical studies, which is endorsed. However, the impact of ADAs on efficacy should also be continued to be evaluated in the ongoing studies. (see also discussion on Clinical Safety)

Pharmacokinetic interaction studies

In vitro studies with cryopreserved human primary hepatocytes indicate that tofersen is not an inducer or inhibitor of CYP450-mediated oxidative metabolism and, therefore, should not compete with other drugs for this metabolic pathway.

Tofersen is not an *in vitro* substrate of BCRP and MDR1 efflux or MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, or OCT2 SLC transporters. Tofersen is also not an in *vitro* inhibitor of MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2 SLC, BCRP, BSEP, and MDR1 transporters. Therefore, the likelihood of DDI due to competition or inhibition of these transporters is very low.

Tofersen is highly bound to human plasma proteins (≥ 95% bound) at clinically relevant or higher plasma concentrations (0.1 and 30 µg/mL). Protein binding in plasma for this class of ASOs is relatively weak, and the binding sites for this type of hydrophilic drug differs from the binding sites of low molecular weight hydrophobic drugs. Therefore, the likelihood of DDI due to competition with plasma protein binding is very low.

2.6.2.2. Pharmacodynamics

Mechanism of action

Tofersen is complementary to a portion of the 3’ untranslated region of the mRNA for human SOD1, binding by Watson-Crick base pairing (hybridisation). This hybridisation of tofersen to the cognate mRNA results in the ribonuclease H-mediated degradation of the mRNA for SOD1, which reduces the amount of SOD1 protein synthesis and accumulation. The binding site is independent from all known SOD1 mutations.

Primary and Secondary pharmacology

Primary pharmacology

CSF SOD1 protein acts as an indirect biomarker of target engagement. A target therapeutic threshold for total CSF SOD1 reduction has not been established. Reductions of at least 20% was targeted as confirmation of target engagement, a threshold beyond the assay and biologic variabilities.

Tofersen administration led to rapid and sustained reductions in total CSF SOD1. A total reduction of SCF SOD1 protein of 35% was observed after 28 weeks of 100 mg tofersen treatment, compared to 2% in the placebo group (difference in geometric mean ratios for tofersen to placebo: 34%; nominal $p<0.0001$). Reductions were apparent by approximately Week 8 and sustained for over 104 weeks of follow-up. However, physiological based PK modelling based on nonclinical data in NHPs suggests that the SOD1 lowering effect of tofersen is unevenly distributed in the CNS with reductions of SOD1 levels of greater than 99% in the spinal cord and of approximately 25% to 30% in the cortex.

In ALS, a lowering of neurofilament levels is generally thought to represent a slowing of axonal injury and neurodegeneration and thus provides evidence of treatment effect. Plasma NfL was evaluated as a key secondary biomarker endpoint in both Studies 101 Part C and 102 and as an exploratory endpoint in Study 101 Parts A and B.

Tofersen administration led to robust and sustained reductions in neurofilament over time. Plasma NfL levels were reduced by 55% (geometric mean ratio to baseline) in tofersen-treated participants, compared to a 12% increase in placebo-treated participants from baseline to Week 28 (difference in geometric mean ratios for tofersen to placebo: 60%; post-hoc nominal $p<0.0001$). Neurofilament levels declined through approximately Week 16 and were sustained over 104 weeks of follow-up.

Secondary pharmacology

The immunogenic response for tofersen in plasma was evaluated in 166 tofersen-treated participants in Studies 101 and 102 with post-baseline plasma samples for ADAs. Overall, including the 15 July 2022 interim data cutoff from Study 102 for participants receiving any tofersen dose, 5 (3.0%) of participants were ADA positive prior to their first dose of tofersen. Ninety-seven (58.4%) tofersen-treated participants developed treatment emergent ADAs, of which 14 were transient and 83 were persistent.

The occurrence of ADA is not expected to produce notable effects on CSF PK since large molecules such as ADAs have limited penetration through the blood brain barrier into the CSF compartment. The impact of ADA was evaluated, but not identified as significant covariate for either the SOD1 protein or NfL PKPD models. Given this, no true impact on clinical function is expected.

A quantitative evaluation of the relationship between tofersen concentration and QTc was performed with results suggesting the absence of a concentration dependent QTcF prolongation. Covariates tested were age, weight, race, sex, baseline QTcF, treatment arm, time of QT collection, administration of concomitant drugs. None of the evaluated covariate relationships were statistically significant ($P>0.01$). In the final analysis, the mean ΔΔQTcF increased with increasing tofersen concentration with a slope of approximately zero. Therefore, the mean and upper limit of the confidence region was below a ΔΔQTcF of 10 ms for the 5^{th} to 95th percentiles of the observed tofersen concentration range.

Relationship between concentration and effect

In popPK modelling the development of a combined model for plasma and CSF was not successful, therefore two separate models were developed. The PK of tofersen in plasma was described using a two-compartment model with depot and first order elimination. Three significant covariates were identified in plasma PK model development ADA on CL, baseline BSA on CL and sex on $V2=V_{central}$. Unexpectedly, positive ADA status was associated with 42.9% reduced clearance, a finding that should be interpreted

with caution. The PK of tofersen in CSF was characterised using a simple one compartment bolus model, no significant covariates were found. The sparse data available (no PK data from phase 3, modelling based on n=166, only trough concentrations) showed high IIV. Both, the plasma, and the CSF compartments were downstream of the CNS, where the site of application and action of tofersen was. Therefore, the findings of the population PK modelling were difficult to interpret. It is agreed that the results of the CSF PK model were only useful to provide an approximation of exposure for the population PKPD models.

Two population PKPD models were developed. For both PD markers indirect response models were used with a maximum effect function. For SOD1 PKPD modelling, 3743 SOD1 protein observations, from 176 patients were used. For NFL PKPD modelling, a total of 2297 observations from 176 participants were included in the final modelling dataset.

The SOD1 pharmacodynamics were described using an indirect response model, where tofersen exposure in CSF was modelled to have an inhibiting effect on the SOD1 entering in the CSF. No covariate effects were found, especially for race this is not regarded as a meaningful result because of the high percentage of patients with white or unknown race. The SOD1 model was used to simulate for dosing of 100 mg, 150 mg, and 200 mg to show that the increase of effect is low using higher dosage than 100 mg. However, the IIV was high, sample size was small (n=176), and data sampling was sparse. Provided results for PKPD modelling suggest a median reduction in CSF SOD1 by about 23 %.

The NfL pharmacodynamics were described as well with an indirect response model used for simulations of dosing for 100 mg, 150 mg, and 200 mg tofersen. No significant covariate effects were found. Also, in CSF-NFL model development there were difficulties regarding convergence of the model, high variability and sparsity of data. Provided results for PKPD modelling suggest a median reduction in plasma NFL of 39 %.

2.6.3. Discussion on clinical pharmacology

Overall, the clinical pharmacology of tofersen has been well characterized.

The provided popPK and PK/PD have a low impact and are considered sufficient to provide information on tofersen PK in humans in the SmPC.

Nevertheless, it should be emphasized that the absence of 1) a joint population PK model (handling both CSF and plasma PK samples) and 2) an exposure-response (safety) analysis, hampers any dosing recommendation (if it exists).

The SmPC is updated with detailed on different PK parameters and exposures as known of today.

2.6.4. Conclusions on clinical pharmacology

In general, the PK of tofersen seem to be sufficiently investigated in CSF and plasma given the sparsity of the available data. The proposed dose is 100 mg IT across patients and no dose adjustments are proposed for different patient populations.

2.6.5. Clinical efficacy

Due to the various analyses from the applicant - two interim analyses with data cut-off 16 January 2022 (week 52) and 28 February 2023 (week 104) - and the Raw Data Pilot project analyses, the structure and sections of Clinical Efficacy as submitted by the applicant have been modified to facilitate the reading flow

The dossier submitted contains only the very minimum number of studies. It is agreed that patients with SOD1 mutation in ALS represent an ultra-orphan disease since they belong to approximately 2% of ALS patients (approximately 1000 prevalent cases in the European Union).

As already pointed out the duration of the pivotal trial Study 101 Part C was short and the population was enriched, and these had consequences in clearly establishing efficacy of tofersen in SOD1-ALS patients.

It is considered that the most appropriate way to summarise the data submitted with this dossier is to present the results for the pivotal phase 3 Study 101 Part C followed by the integrated/combined results of studies 101 Part C and long term extension (LTE) 102 (with the results of two interim analyses with data cutoff after 52 weeks on 16 January 2022 and after 104 weeks 28 on February 2023).

There are three interim analyses relevant for efficacy with data cutoff on:

16 July 2021- 1st Interim analysis

16 January 2022 – 2nd Interim analysis

28 February 2023 – 3rd Interim analysis (CHMP requested)

2.6.5.1. Dose response study(ies)

Dose levels evaluated in clinical trials were informed by nonclinical toxicology studies, iterative translational predictions, and clinical trial experience.

Study 101 was a randomized, double-blind, placebo-controlled study to examine the efficacy, safety, tolerability, PK, and PD of tofersen administered to participants with SOD1-ALS. Initially designed as a Phase 1 single ascending dose (SAD) (Part A) and multiple ascending dose (MAD) (Part B) study.

Study 101 Part A (SAD) was a randomized, double-blind, placebo-controlled evaluation of 4 dose levels of tofersen (10, 20, 40, and 60 mg) administered to participants with SOD1-ALS. In total, 15 participants received a single dose of tofersen, and 5 participants received placebo. Participants were followed for approximately 8 weeks in the study and were eligible to be screened for Part B (MAD) thereafter. Participants who did not meet eligibility criteria for Part B were offered the opportunity to enrol in the ongoing open-label study, Study 102 LTE.

Study 101 Part B (MAD) was a randomized, double-blind, placebo-controlled evaluation of 4 dose levels of tofersen (20, 40, 60, and 100 mg) administered for approximately 12 weeks (3 loading doses on Days 1, 15, and 29 plus 2 maintenance doses on Days 57 and 85) in participants with SOD1-ALS. In total, 50 participants were randomized to receive tofersen (n = 38) or placebo (n = 12) and were followed for approximately 24 weeks before having the opportunity to enrol in Study 102.

Study 101 Part B was originally designed to test 3 dose levels of tofersen (20, 40, and 60 mg). PK data from the 39-week NHP toxicology study indicated that higher exposure to tofersen would effectively reduce CSF SOD1 levels. A fourth dose level of tofersen (100 mg) was added by amendment.

In total, 50 participants were enrolled in the MAD portion of Study 101 (Part B), of whom 12 received placebo and 10 received tofersen 100 mg. Consistent with the proportion of participants that met the fast progressor criteria defined in the statistical analysis plan (SAP), baseline disease progression characteristics were most balanced in the tofersen 100 mg and placebo groups thus, enabling comparison.

IT administration of tofersen 100 mg in Study 101 Part B led to the highest tofersen exposures, the greatest reduction in total CSF SOD1 protein, and an apparent slowing of decline across several exploratory clinical outcome measures compared with lower doses. Physiological based PK modelling based on nonclinical data in NHPs suggests that tofersen 100 mg achieves exposures in CNS that are likely to reduce SOD1 levels by greater than 99% in the spinal cord and by approximately 25% to 30% in the cortex.

The PK of tofersen is summarized as follows. Maximum CSF concentrations of tofersen are achieved rapidly after IT administration. Steady-state concentrations in CSF were achieved immediately after the 4-week loading dose period. Minimal accumulation of tofersen in CSF was observed after the loading dose period. The effective $t_{1/2}$ of tofersen in CSF was approximately 1 month. Although CNS tissue $t_{1/2}$ cannot be measured in humans, the terminal $t_{1/2}$ was measured in the CNS tissue of cynomolgus monkeys and found to be 31 to 40 days. This suggests that the CSF and CNS tissue concentrations likely reached equilibrium during the post-distribution period. Because the site of action of tofersen is within the CNS, these findings support the proposed frequency of dosing and route of administration.

Tofersen is rapidly transferred from CSF into the systemic circulation, with a median time to reach maximum observed concentration of 2 to 6 hours. After reaching the peak levels, plasma concentrations of tofersen declined rapidly, likely due to extensive distribution to systemic tissues.

Recognizing that SOD1-ALS is thought to be a predominantly lower motor neuron disease and a substantially higher dose level would be required to achieve a meaningfully differentiated SOD1 reduction in the cortex, the 100 mg dose level was selected for evaluation in the Phase 3 study (101 Part C). Specifically, tofersen 100 mg was administered as 3 loading doses at 14-day intervals on Days 1, 15, and 29, followed by maintenance doses every 28 days thereafter.

Based on the results described in the application, the proposed commercial dosage regimen is identical to the regimen investigated in Study 101 Part C (CSR 233AS101 Part C), i.e., 3 loading doses of tofersen 100 mg once every 2 weeks, followed by maintenance doses once every 4 weeks administered via IT bolus injection.

In CSF there was a less than proportional increase of tofersen exposures with increasing doses from 20 to 100 mg. The tofersen CSF concentration in participants receiving tofersen 10 mg was below the limit of quantitation at all sampling timepoints. Concentrations were highest in the 100 mg group, lowest in the 20 mg group, and similar in the 40 and 60 mg groups. Concentrations of the 100 mg dose were approximately 2 to 3 times higher than those observed for the 20 mg dose. However, tofersen plasma PK showed significant variability and formal PK linearity analysis was not performed due to the small sample size. However, there seemed to be a roughly dose-proportional increase in C_{max} and AUC values over the evaluated dose range. The plasma PK parameter values were generally consistent between the first and last doses.

2.6.5.2. Main study(ies)

The applicant has completed study 233AS101, consisting of phase 1/2 parts A and B and phase 3 part C. Part C of study 101 is intended to support the efficacy and safety of tofersen together with the open-label extension (OLE) or LTE study 233AS102 (further referred to as study 102).

In the following sections, each study's methods and results are presented separately. The pivotal controlled study, study 101 Part C will be presented first and then the open-label Study 102 LTE afterwards. The analyses across studies 101 and 102 will be also summarised.

2.6.5.2.1. Study 101 Part C (up to week 28)

Methods

Study 101 Part C was a Phase 3, randomized, double-blind, placebo-controlled study designed to assess the efficacy and safety of tofersen 100 mg versus placebo over 6 months. Initially designed to enrol 60 participants and subsequently amended to enrol 99 participants following regulatory feedback, a total of 108 participants with weakness attributable to ALS and a confirmed *SOD1* mutation were randomized 2:1 to receive tofersen or placebo for approximately 24 weeks (3 loading doses followed by 5 maintenance doses).

- **Study Participants**

A total of 108 participants were randomized (tofersen 100 mg, n = 72; placebo = 36), of whom 60 participants met prognostic enrichment criteria for faster disease progression and were included in the modified intent-to-treat (mITT) population (tofersen 100 mg = 39; placebo = 21). The prespecified primary analysis population, or mITT population, comprised the subset (n = 60) of participants who met the prognostic enrichment criteria for rapid disease progression based on their SOD1 mutation type and prerandomisation Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) slope (also referred to as the enriched or faster progressing/faster progressor subgroup - Table 4). All other participants (n = 48) were classified as the non-mITT population (also referred to as the other or slower progressing/slower progressor subgroup). As such, only descriptive analyses were performed, except for total CSF SOD1, which was formally tested as the primary endpoint in the non-mITT population.

Table 4: Protocol-defined disease progression subgroups

	Faster-Progressing Subgroup ("enriched"; mITT)	**Slower-Progressing Subgroup ("other"; non-mITT)**
Mutation type and prerandomisation ALSFRS-R slope	Protocol-defined *SOD1* mutation historically associated with shorter survival[a] and ≥ 0.2 points/month prerandomisation slope OR Another *SOD1* mutation and **≥** 0.9 points/month prerandomisation slope	Another *SOD1* mutation and < 0.9 points/month prerandomisation slope
SVC cutoff	≥ 65% predicted	≥ 50% predicted

a p.Ala5Val, p.Ala5Thr, p.Leu39Val, p.Gly42Ser, p.His44Arg, p.Leu85Val, p.Gly94Ala, p.Leu107Val, and p.Val149Gly

Study 101 Part C enrolled adults with weakness attributable to ALS and a confirmed SOD1 mutation.

SOD1 mutation must have been confirmed by the central reader based on the sample obtained during the Screening Visit; participants with an SOD1 mutation interpreted by the central reader to be pathogenic or likely pathogenic were eligible.

Concomitant riluzole and/or edaravone use was permitted for participants who were on a stable dose for at least 30 or 60 days prior to study baseline, respectively. In the overall intent-to-treat (ITT), approximately 62% of participants were receiving riluzole and 8% were receiving edaravone at baseline.

Study 101 was a Phase 1/2/3 study evaluating the efficacy, safety, tolerability, PK, and PD of tofersen administered to adult participants with ALS and confirmed SOD1 mutation. The applicant has chosen to modify the ITT population based on the prerandomisation ALSFRS-R slope. It is not considered appropriate to create ITT subgroups in a population which is already a proportion of ALS patients.

However, due to the heterogeneity of the SOD1 ALS population it can be justified, as long as it is pre-specified.

- **Treatments**

Tofersen (BIIB067) was supplied as a liquid in vials containing 6.7 mg/mL of BIIB067. Artificial CSF was used as placebo. A total of 8 lots of tofersen and placebo were used.

A total of 100 mg of tofersen or placebo was administered 8 times (3 loading doses once every 2 weeks and 5 maintenance doses once every 4 weeks) by IT administration. A total volume of 15 mL tofersen or placebo was administered over a 1- to 3-minute bolus injection.

Study duration was approximately 32 to 36 weeks:

- 4-week screening period
- 24-week treatment period
- 4- to 8-week follow-up period as follows:
    - Participants who enrolled (uninterrupted) in the long-term extension (LTE) study (233AS102), the Week 28 Visit served as the end of study (EOS) Visit.
    - Participants with delayed enrolment in the LTE study, the Week 32 Visit served as the EOS Visit (either in person or by telephone contact).

▪ If enrolment in the LTE occurred ≤ 28 days from the planned Week 28 Visit, the Week 32 Visit (EOS) assessments were conducted at the time of enrolment in the LTE.

▪ If enrolment in the LTE occurred > 28 days from the planned Week 28 Visit, the Week 32 Visit (EOS) assessments were conducted as planned (4 weeks from the planned Week 28 Visit).

    - Participants who did not enrol in the LTE study, the Week 32 Visit served as the EOS Visit (either in person or by telephone contact).

The treatment regimen of tofersen has been selected using PK modelling data and the data from study Parts A and B. The use of placebo as a comparator is appropriate as no product has been approved for the treatment of SOD1-ALS.

Storage

Tofersen and placebo were to be stored at 2°C to 8°C and protected from light.

Missed doses

No description of handling of missed doses was described in the protocol of Study 101 Part C.

- **Objectives**

The primary objective was to evaluate the clinical efficacy of tofersen administered to adult participants with ALS and a confirmed SOD1 mutation.

- **Outcomes/endpoints**

The primary endpoint was change from baseline to Week 28 in ALSFRS-R total score.

Key secondary endpoints in order of hierarchical testing

- Change (i.e., ratio) from baseline in total SOD1 protein concentration in CSF
- Change (i.e., ratio) from baseline in neurofilament light chain (NfL) concentration in plasma
- Change from baseline to Day 197(Week 28) in percentage predicted slow vital capacity (SVC)
- Change from baseline to Day 197 in hand-held dynamometry (HHD) megascore

- Time to death or permanent ventilation (PB) (defined as ≥ 22 hours of mechanical ventilation [invasive or non-invasive] per day for ≥ 21 consecutive days)
- Time to death

Analytically, the following endpoints were measured for efficacy:

- ALSFRS-R
- SVC
- HHD megascore
- Participant diary/eDiary to record ventilation use
- Time to death
- Motor unit number index
- Quality of life as measured by the following: Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-5), Fatigue Severity Scale (FSS), European Quality of Life Five Dimension Five Level Questionnaire (EQ-5D-5L), Work Productivity and Activity Impairment (WPAI), Zarit Burden Interview (ZBI), 36-Item Short Form Health Survey (SF-36), Patient Global Impression of Change, Clinician Global Impression of Change, Patient Global Impression of Status (PGIS), and Clinician Global Impression of Status.

Pharmacodynamics:

- total SOD1 protein in CSF

Biomarkers*:

- Misfolded or mutant SOD1
- NfL [blood (serum) and CSF]
- pNfH [blood (plasma, serum) and/or CSF]
- Urinary p75 extracellular domain (p75ECD)

* The following biomarker data were not available at the time of writing of the CSR due to assay availability: urinary p75ECD and misfolded or mutant SOD1. Both NfL and pNfH were measured in CSF and plasma, not in serum.

Pharmacokinetics:

- Tofersen concentrations in the plasma and CSF were determined using validated assays.

The functional scales as well as the patient reported outcomes (PRO) selected for the conduct of the study are considered appropriate. The choice of the time-point for the end of study measurements, week 28, is considered too short to show any potential efficacy of tofersen. This issue has been extensively discussed during the scientific advice procedures.

- **Sample size**

For Study 101 Part C, the sample size was selected primarily based on the JRT combining the Week 28 change from baseline in ALSFRS-R total score and mortality in the mITT population.

Interim data from Study 101 Part B in the fast-progressor group as well as the observed ALSFRS-R decline in a similar fast-progressor SOD1-ALS population receiving placebo from a historical data set (Benatar 2018) are the basis of the assumed treatment effect and variability according to the applicant.

Participants from both datasets were matched with the prognostic enrichment criteria for rapid disease progression specified in Protocol 233AS101 Version 8. Based on these 2 datasets to determine the expected ALSFRS-R decline and limited survival data in fast-progressors from the literature on participants with a SOD1 A5V mutation, the sample size calculation assumed a 24.7-point decline in the placebo group and a 4.8-point decline in the tofersen group over 28 weeks, a pooled SD of 3.166, and expected survival of 82% in the placebo group and 90% in the tofersen 100 mg group. The assumptions for overall survival were taken from overall survival data in fast-progressors from the literature on individuals with a SOD1 A5V mutation. With 60 participants in the mITT population and a two-sided significance level of 0.05, the JRT would provide 84% power. Because events of death were factored into

the analysis, no sample size overage was planned for missing values. For the population outside the mITT population, the primary focus was on the PD endpoint, total CSF SOD1 protein concentration, as this population was anticipated to have a slower decline in clinical function than the mITT population. A sample size of 26 participants in the tofersen-treated group and 13 participants in the placebo group for the population outside of the mITT would provide 97% power to detect a 25% reduction in total CSF SOD1 protein from baseline in the tofersen-treated group, with an assumed SD of 0.216 (natural log scale), compared with the placebo group. A 25% reduction in the CSF SOD1 protein concentration was estimated to exceed both the SOD1 assay variability and the longitudinal biovariability of CSF SOD1 as measured in humans.

Therefore, Part C was planned to randomize a total of approximately 99 participants, with participants receiving tofersen and placebo in a 2:1 ratio. The COVID-19 mitigation plan lowered the risk of missed doses and assessments and was in place to avoid the need for a sample size increase.

However, rather than the anticipated 24.7-point decline on the ALSFRS-R, the placebo arm declined by 8.1 points over the 6-month period, suggesting that the relatively short trial duration limited the amount of time to overcome disease heterogeneity in the population.

- **Randomisation and Blinding (masking)**

Participants were randomized to receive tofersen 100 mg or placebo in a 2:1 (active: placebo) ratio for 28 weeks (197 days) at 32 sites globally. Randomisation was stratified by 3 factors: 1) whether a participant met prognostic enrichment criteria for rapid disease progression; 2) whether a participant used edaravone at baseline; and 3) whether a participant used riluzole at baseline.

With respect to blinding (masking) in Study 101, treatment assignments were blinded to applicant, vendors, participants, caregivers, and study site personnel involved in the assessment of efficacy and safety. To preserve this blind, Study 102 was designed with a blinded loading dose period in which those who previously received tofersen in Study 101 received a dose of placebo at Day 15, and vice versa.

Unblinded safety data for Studies 101 Part C and 102 were reviewed on a quarterly basis by the independent data safety monitoring committee (IDMC). An independent biostatistician and statistical programmer prepared the unblinded outputs and sent these via a secure method to the IDMC.

Though elevations in CSF white blood cells have been observed in both placebo and tofersen-treated participants, these elevations have been observed at a greater frequency and to a greater magnitude with tofersen treatment. In an abundance of caution, access to participant-level CSF related laboratory data was restricted from blinded applicant team members in the clinical database. Measures implemented are detailed in the Unblinding Plan. Designated ALSFRS-R raters were not involved in any other aspect of the study and remained blinded to results of all other study assessments.

These measures were implemented for both Studies 101 Part C and 102, and they continue to be maintained for the ongoing Study 102.

Following the study completion and database lock of Study 101 Part C, a small team at applicant´s side was unblinded to treatment assignments to prepare this application, while an independent blinded team continued to work on the ongoing study management activities for Study 102. This study team overseeing Study 102 remains blinded to treatment assignments, as do all vendors, participants, caregivers, investigators, and study site personnel. The unblinded team were not involved in the study management and collection of data for the ongoing Study 102 following the initial interim lock based on the 16 July 2021 data cut. On conducting a second interim analysis, the unblinded team had no access to the data collected between the 16 July 2021 data cut and the 16 January 2022 data cut until after the second interim lock occurred, at which time they were given access to all data up to this data cut. The unblinded team were responsible for the modified SAP which was finalized prior to the interim lock based

on the data cut of 16 January 2022. All these measures continue to be maintained. Post-withdrawal vital status data for participants who withdrew from Studies 101 Part C or 102 were collected retrospectively in a blinded manner by the blinded study team, while the unblinded team have no access to these data.

- **Statistical methods**

Estimand (target of estimation)

The estimand of the primary analysis was defined as follows:

- Population: all participants in the mITT population.
- Variable: change from baseline to Day 197 in the ALSFRS-R total score.
- Intercurrent events: relevant intercurrent events (death and withdrawals) were handled using a composite strategy in which participants who had these intercurrent events were ranked against each other and against participants without any intercurrent event using the JRT methodology based on MI datasets. Participants were ranked using their Day 197 value (or imputed Day 197 value for withdrawals).
- Summary statistics: difference between treatment groups in least squares (LS) means of Day 197 change from baseline with corresponding standard errors (SEs) and 95% confidence intervals (CIs) taken from the ANCOVA for change from baseline to Day 197, based on the MI dataset.

The definition of the estimand is not compliant with ICH E9(R1) and is overall inconsistent. The estimand describes the target of estimation (scientific question of interest) in alignment with the objective of the study. Statistical methods need to be aligned to the estimand definition but are not part of the estimand definition.

The intercurrent events 'death' and 'withdrawal' are claimed to be handled by a composite strategy. However, according to the description of the applied JRT+MI method only deaths were actually handled in alignment with a composite strategy (by assigning worst ranks according to survival time), while imputing missing values for withdrawals under a missing at random (MAR) assumption is not aligned to a composite strategy but to the hypothetical strategy 'if the patient had not withdrawn'.

The ANCOVA+MI for estimation of the population level summary measure is also aligned to a hypothetical strategy not only for withdrawal but also for death and is therefore not consistent with the description of the strategies for the intercurrent events.

However, for treatment withdrawals, the treatment policy strategy (i.e. the effect irrespectively of withdrawal) is usually of regulatory interest as withdrawal from treatment may be related to effects of the treatment. For the intercurrent event death, a composite strategy is appropriate. However, in case of ALSFRS-R as the variable of interest, the value '0' can also be considered as the true value as death can be considered as complete loss of function.

Statistical methods

The SAP describes in detail the planned statistical methods for data collected in Part C.

Analysis populations:
ITT population: all subjects in Part C who were randomized and received at least 1 dose of study treatment.

Modified ITT Population: all subjects who meet the prognostic enrichment criteria for rapid disease progression in Part C who were randomized and received at least 1 dose of study treatment.

Non mITT Population: the subset of subjects in the overall ITT population who were not included in the mITT population, i.e. all other eligible subjects.

For each of the primary efficacy and secondary efficacy endpoints, the key analysis used for formal testing was in the mITT population and was classified as the "primary analysis" for that efficacy endpoint. Analyses in the non-mITT population and overall ITT population of each of the primary and secondary endpoints were also conducted and were classified as secondary analyses, with the exception of total SOD1 protein in CSF, which was the primary endpoint for the non-mITT population.

The primary efficacy endpoint in Study 101 Part C was change from baseline to Week 28 in ALSFRS-R total score in the mITT population. The primary efficacy analysis was a Joint Rank Test (JRT) in conjunction with multiple imputation (MI) to handle withdrawals for reasons other than death. MI was used to impute all missing data before determining the rank score, including data after withdrawal from the study except after death. The MI model for the mITT population included treatment, riluzole or edaravone use, baseline score, and postbaseline scores. Subjects who died were given lower ranks than subjects who completed the study or withdrew from study for other reasons than death, with the lowest ranks being given to the subjects who died in the shortest time after first dose. For subjects who completed study or discontinued for other reasons than death, progressively higher ranks were given to subjects with a higher change from baseline at Day 197 (i.e. smaller decline at Day 197). For each of the imputed datasets, the ranked scores were analysed using an ANCOVA model with treatment included as a fixed effect and adjusted for the following covariates: baseline disease duration since symptom onset, baseline ALSFRS-R total score, and use of riluzole or edaravone. The model was then used to obtain the p-value based on Rubin's rule from PROC MIANALYZE for the joint rank. The treatment comparison was based on a two-sided alpha level of 0.05.

As summary statistics for the treatment effect, the difference between treatment groups in LS means of Day 197 change from baseline with corresponding SEs and 95% CIs taken from the ANCOVA+MI for change from baseline ALSFRS-R total score to Day 197, replacing missing values after withdrawal, intermittent missing data and after death based on the MI model described above. The ANCOVA model included treatment group as a fixed effect and covariates baseline ALSFRS-R total score, baseline disease duration since symptom onset, and use of riluzole or edaravone. The corresponding nominal p-value was also presented for the ANCOVA+MI.

Change from baseline to Week 28 (Day 197) in percent predicted SVC for the mITT population was analysed as for the primary efficacy endpoint.

Changes from baseline to Week 28 (Day 197) i.e., ratios to baseline in each of total CSF SOD1 protein, plasma NfL and HHD megascore were analysed for the mITT population using ANCOVA with MI. Total CSF SOD1 protein was also analysed similarly in the non-mITT population. Data for PD/biomarker endpoints were log transformed for the ANCOVA analysis with MI.

Ventilation assistance-free survival and overall survival, respectively, were analysed as time to event endpoints. Kaplan-Meier estimates of the cumulative probability of event occurrence over time were determined. Treatment comparison was based on a log-rank test stratified by treatment and riluzole or edaravone use. A Cox regression model adjusted for baseline disease duration since symptom onset, and riluzole or edaravone use was used to obtain the hazard ratio and 95% CIs for each of these endpoints.

If the primary endpoint was statistically significant at the two-sided alpha of 0.05, the analyses of secondary endpoints were based on a sequential testing procedure in the order of the rank of secondary endpoints as listed in the endpoint section.

Among several sensitivity analyses to assess the robustness of the primary efficacy analysis, given the potential utility of neurofilament as a biomarker of ALS disease activity, baseline plasma NfL was included as an additional covariate in ANCOVA model for both ranked scores and change from baseline to Day 197 in ALSFRS-R total score.

Post-hoc analyses

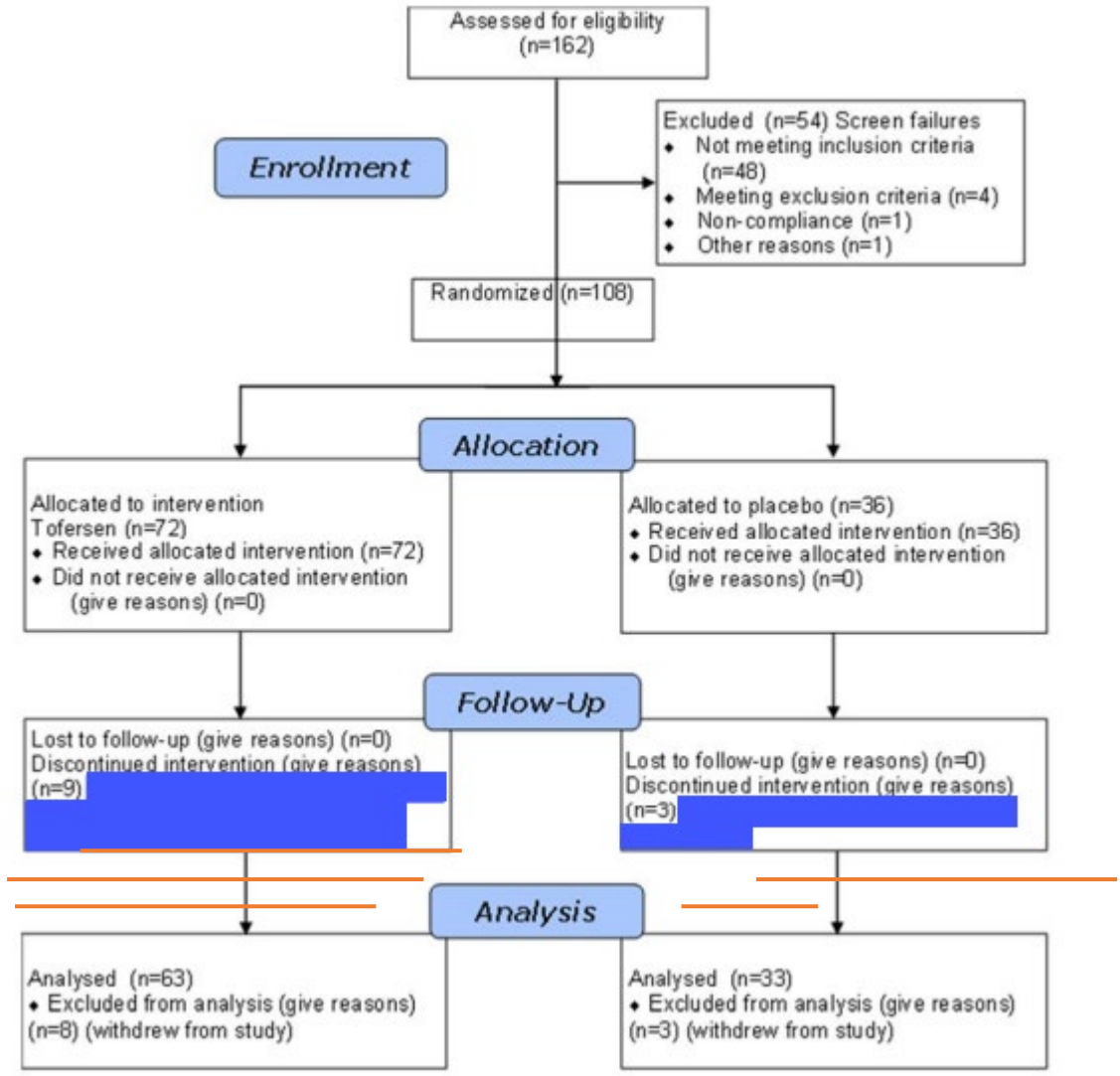
An ANCOVA analysis was performed for the overall ITT population; it included treatment as a fixed effect and was adjusted for the following covariates: baseline plasma NfL (in original scale), riluzole/edaravone use, and the corresponding baseline value for the endpoint. Disease duration since symptom onset was replaced by baseline plasma NfL because this is a better predictor of disease progression. In addition, for SVC, baseline plasma NfL replaced both duration since symptom onset and baseline ALSFRS-R score, which had originally been specified as prognostic factors for disease progression.

The following analyses were specified for the ISE but also provided for Study 101, Part C: Disease progression subgroup analyses (mITT/non-mITT and < median, ≥ median baseline NfL) and ITT population analyses include post hoc analyses adjusting for both disease duration and baseline plasma NfL, analyses adjusting for ALSFRS-R prerandomisation slope, analyses adjusting for ALSFRS-R prerandomisation slope and baseline plasma NfL.

Results

- **Participant flow**

Figure 7: Participants flow and disposition of screened participants for study 101 Part C



Reasons for discontinuation may include adverse event, consent withdrawn, non-compliance with study protocol, disease progression and others. The specific reasons per arm are removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

- **Recruitment**

  Date of first treatment: 27 March 2019 - End of Study Date: 16 July 2021

  Approximately 99 participants were planned to be randomized. A total of 108 participants were randomized and dosed, with 72 participants receiving tofersen and 36 participants receiving placebo. A total of 97 participants completed the study. One participant discontinued treatment arm due to pulmonary embolism but continued in the study.

- **Conduct of the study**

The original protocol, dated 24 September 2015, was amended 8 times (the current protocol is Version 8), with an additional 24 country-specific amendments (CSAs). Part C was added to Study 233AS101 as part of Version 5 (20 Dec 2018); this amendment and all subsequent amendments are described in the table below. Protocol amendments Version 2 through Version 4 are described in the separate Part A/B case study report because they predated the addition of Part C to the study.

Overall, in the ITT population, 88.9% of participants (96/108 participants) had a major protocol deviation. The most common deviations were in the categories of efficacy criteria (63.0%, 68/108 participants), study procedures criteria (32.4%, 35/108) and laboratory assessments (30.6, 33/108 participants). Major protocol deviations due to COVID-19 were reported in 33.3% of participants (36/108 participants). The most frequently reported reason for major COVID-19–related protocol deviations included movement restrictions related to COVID-19 pandemic (11.1%), other COVID-19–related reason (11.1%), and sites closed or access restricted due to the COVID-19 pandemic (9.3%)

Multiple procedures were implemented across studies to ensure data integrity. Study personnel underwent standardized training on the protocol and study assessments prior to the enrolment of participants at their institution. To preserve the integrity of data, site staff, study participants, and vendors remain blinded to individual treatment assignments from Study 101 Part C.

There were some major amendments to the protocol with the most important one being the change of the analyses focusing on the NfL levels and not the pre-randomisation slope. This change took place just before the interim database lock 2 for Study 102 in February 2022. Thus, it cannot be considered as "Prespecified" in terms of the initial protocol and prior to the final database lock and unblinding. It was *post-hoc* for Study 101 Part C and although it was specified at a later time-point and before the analysis of study 102, changes were made in knowledge of results from the ongoing study because study 102 as an extension study is not independent from Study 101 Part C.

Major protocol deviations were observed but these were similar between the "enriched" subgroup and the other. A slightly higher percentage of the overall protocol deviations were recorded in the tofersen group (91.7%) compared to the placebo group (88.3%) in the ITT population. However, for the deviations categorised under "efficacy criteria" the percentages among groups were similar 62.5 % for tofersen and 63.9% for placebo. Since differences were not observed, an impact of the deviations on the outcome of this double-blind randomised study is not expected.

- **Baseline data**

Applicant's analyses and presentation

Most participants were White (63.9%), followed by Asian (8.3%). A large proportion of participants did not report their race and ethnicity (25.9%) due to confidentiality regulations. The percentage of males and females was similar across treatment groups and populations, with more males enrolled overall (males, 57.4% and females, 42.6%). Participant ages ranged from 23 to 78 years with a higher mean participant age in the placebo group in the "enriched" subgroup (54.0 years) compared with the tofersen group (47.3 years). Other demographic characteristics were generally balanced between treatment groups and populations.

Baseline demographic and most disease characteristics (Table 5) were balanced across treatment arms for use of riluzole and/or edaravone and characteristics related to the stage of disease, including time from onset, total ALSFRS-R score, and SVC. Higher levels of NfL were observed in the tofersen group suggesting potentially faster disease progression compared to the placebo group.

The population enrolled in this study was representative of the broad SOD1-ALS population. Forty-two unique SOD1 mutations centrally confirmed as pathogenic or likely pathogenic were enrolled. The most commonly identified SOD1 mutation types were p.Ile114Thr (n= 20/108; 18.5%), p.Ala5Val (n = 17/108; 15.7%) and p.Gly94Cys (n = 6/108; 5.6%), and p.His47Arg (n = 5/108; 4.6%). It is noted that A4V/A5V (p.Ala5Val) mutation carriers are considered the most prevalent fast progressing mutation type enrolled which is consistently associated with a median survival (based on Kaplan Meier) at or below 1.2 years.

Table 5: Baseline disease characteristics

	mITT (n=60)		**non-mITT (n=48)**		**ITT (n=108)**	
	placebo (n=21)	**tofersen 100 mg (n=39)**	**placebo (n=15)**	**tofersen 100 mg (n=33)**	**placebo (n=36)**	**tofersen 100 mg (n=72)**
Mutation[1] type n (%)						
p.Ile114Thr	6 (**29**)	5 (**13**)	4 (27)	5 (15)	10 (**28**)	10 (**14**)
p.Ala5Val	6 (29)	11 (28)	0	0	6 (**17**)	11 (**15**)
p.Gly94Cys	1 (5)	1 (3)	1 (7)	3 (9)	2 (6)	4 (6)
p.His47Arg	0	0	4 (27)	1 (3)	4 (11)	1 (4)
Site of onset n (%)						
Bulbar	2 (10)	3 (8)	N	N	3 (8)	3 (4)
Lower limbs	14 (67)	19 (49)	12 (80)	27 (82)	26 (72)	46 (64)
Upper limbs	5 (24)	14 (36)	2 (13)	6 (18)	7 (19)	20 (28)
Respiratory	N	N	0	0	N	N
Multiple sites	N	N	0	0	N	N
Time from symptom onset (months) median (min, max)	**8.3** (2.4, 21.3)	**8.3** (1.7, 18.5)	**39.6** (11.8, 103.2)	**35.5** (3.9, 145.7)	**14.6** (2.4, 103.2)	**11.4** (1.7, 145.7)
ALSFRS-R pre-randomisation slope: median (min, max)	**-1.51** (-4.91, -0.42)	**-1.34** (-8.30, -0.39)	**-0.17** (-0.84, -0.02)	**-0.30** (-0.77, 0.00)	**-0.89** (-4.91, -0.02)	**-0.75** (-8.30, 0.00)
ALSFRS-R baseline total score: mean (SD)	**35.4** (5.66)	**36.0** (6.40)	**39.9** (5.09)	**38.1** (5.13)	**37.3** (5.81)	**36.9** (5.91)
Range: min, max	24, 45	15, 44	32, 47	26, 48	24, 47	15, 48
ALSFRS-R run-in slope (Screening to Day 15) raw mean (SD)	-1.3 (3.91)	-1.8 (2.47)	0.1 (1.87)	-0.1 (1.34)	-0.7 (3.25)	-1.0 (2.19)
% predicted SVC at baseline: mean (SD)	83.7 (17.87)	80.3 (14.22)	87.1 (14.82)	84.2 (19.02)	**85.13** (16.53)	**82.1** (16.59)
Range: min, max	57.4, 120.4	46.7, 114.8	54.8, 114.4	55.4, 134.7	54.8, 120.4	46.7, 134.7
Plasma NfL at baseline (pg/mL) mean (SD)	**127.3** (94.4)	**146.2** (82.6)	**37** (29.5)	**47.6** (41.8)	**89.7** (86.5)	**100.4** (82.8)
Geometric mean Range: min, max	92.7 9, 370	121.8 12, 329	28.4 8, 99	33.2 5, 211	56.6 8, 370	66.6 5, 329

1 Most common mutations, i.e., n > 4
N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

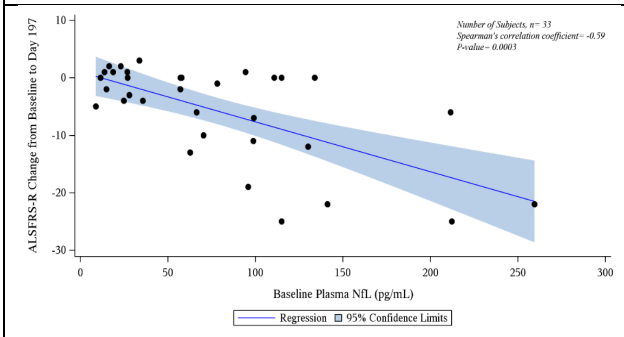
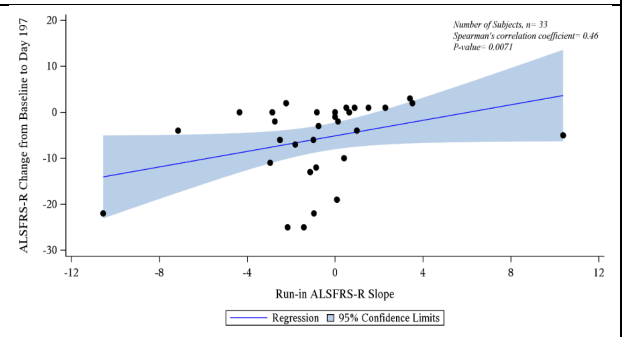
In the ITT population mean values for ALSFRS-R baseline total score and % predicted SVC at baseline were slightly worse in the tofersen group with 36.9 and 82.1% respectively, compared to the placebo group with values of 37.3, and 85.13% and, respectively. The geometric mean baseline plasma NfL for the prespecified mITT population was approximately 29 pg/mL (23%) higher in the tofersen group (122

± 83 [standard deviation, SD] pg/mL) than in the placebo group (93 ± 94 [SD] pg/mL). Baseline NfL levels, which have been shown to correlate with disease progression, were approximately 15% to 25% higher in the tofersen group (100.4 pg/ml) than in the placebo group (89.7 pg/ml) in the whole ITT population. Consistently, the rate of decline on ALSFRS-R from screening to Day 15 (approximate 42-day period) was greater in the tofersen group compared to the placebo group. Together with the duration of symptom onset (median 14.6 months for placebo vs median 11.4 months for tofersen group) this may indicate more stable disease in patients in the placebo group. According to the applicant, these imbalances are apparent in the ITT population as well as the protocol-defined subgroups. Subgrouping the population by baseline plasma NfL levels (rather than mutation type and pre-randomisation ALSFRS-R slope decline) appeared to correct these imbalances between the tofersen and placebo groups.

To account for the significant disease heterogeneity in SOD1-ALS and the relatively short study duration of Study 101 Part C, the applicant had implemented protocol-defined prognostic enrichment criteria for the mITT population.

However, consistent with the literature (Thompson 2022), baseline neurofilament levels were more strongly correlated with longitudinal change in ALSFRS-R (Spearman correlation coefficient: -0.59; p = 0.0003) than was prerandomisation ALSFRS-R slope decline (Spearman correlation coefficient: 0.46; p = 0.0071) in the placebo participants in Study 101 Part C (Figure 8).

Figure 8: Baseline plasma NfL outperformed prerandomisation ALSFRS-R decline as a predictor of 6-month change in ALSFRS-R in Study 101 Part C placebo participants

Baseline plasma NfL (correl. coeff. -0.59)	Run-in ALSFRS-R slope (correl. coeff. 0.46)
	

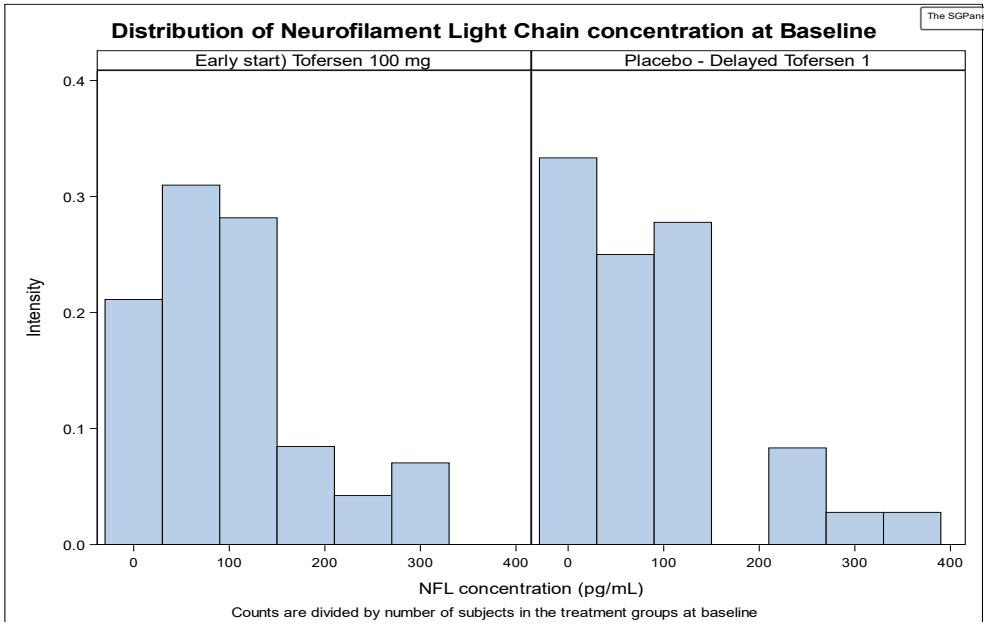
Baseline characteristics (SOD1 CSF protein levels)

The mean concentration of total SOD1 protein in the CSF was balanced between placebo and tofersen groups at baseline in the mITT population (117.2 ng/mL and 118.1 ng/mL, respectively), and slightly lower in the tofersen group than in the placebo group for the non-mITT population (120.4 ng/mL versus 135.8 ng/mL) and ITT population (118.7 ng/mL versus 125.5 ng/mL).

Raw Data Pilot Project analyses and presentation

The Figure below shows the distribution of NfL concentration (pg/ml) at baseline in the two treatment groups. X-axis indicates NfL concentration in pg/ml and y-axis indicates proportion of subjects, i.e. counts of subjects with a NfL assessment in the indicated group divided by the number of subjects in the treatment groups at baseline (see below at Figure 9 the 1st and the 2nd evaluation phase of the Raw Data Pilot project).

Figure 9: Distribution of neurofilament light chain concentration (pg/ml) at baseline



With respect to mutation types, all participants had a centrally confirmed pathogenic or likely pathogenic SOD1 mutation, as adjudicated by the central laboratory, with 42 unique SOD1 mutations. The most commonly identified SOD1 mutation types (> 10% of participants) were p.Ile114Thr (n=20/108; 18.5%) and p.Ala5Val (n=17/108; 15.7%). It would have been worthwhile to further explore the results with patients with certain variants or mutation types such as A4V/A5V (p.Ala5Val), but its small number in the study (17 out of 108) would not allow any meaningful conclusions. In addition, it appears that in most cases there are only 1 or 2 patients per variant, which is not helpful for subgroup analysis.

It is noted that the natural history of SOD1-ALS is highly variable with a mean onset of 49.7 ± 12.3 years and mean disease duration of 4.6 (1.4 for A4V and 6.6 for non-A4V) years. Disease progression for individual mutations is variable, with survival ranging from less than a year to over 20 years (Bali 2017; Cudkowicz 1997).

It should be also noted that recently a study with a cohort of long survivors in Scotland was published (Leighton et al 2022). Recent analysis of a historical cohort of 428 Scottish people with motor neuron disease indicated a median survival of 3.5 years from onset of symptoms and 2 years from diagnosis. However, the upper range of survival was 25.8 years from diagnosis. Fifty-eight long survivors with MND were identified in Scotland. Median survival from diagnosis was 15.5 years. Long survivors were significantly younger at onset and diagnosis than incident patients and had a significantly longer diagnostic delay. 42% had the motor neuron disease subtype of primary lateral sclerosis. Whole genome sequencing was performed in 46 individuals: 14 (30.4%) had a potentially pathogenic variant. Four carried the known SOD1 p.(Ile114Thr) variant (Leighton et al 2022). From these 4 survivors, 2 had a mean survival from onset in months (years): 199 (16.6), one 232 (19.3) and one 263 months (21.9). The authors suggested that for example the absence of a C9orf72 pathogenic expansion and presence of the SOD1 p.(Ile114Thr) mutation or other rare mutation might indicate better outcome.

- **Numbers analysed**

Applicant's analyses and presentation

A total of 97 participants completed the study. Treatment discontinuations and study withdrawals were similar in the tofersen group compared to the placebo group; treatment discontinuations and study withdrawals were more common in the "enriched" subgroup. The most common reason for

discontinuation of study treatment was disease progression (n = 5, 4.6%), all of which occurred in the "enriched" subgroup, followed by adverse events (AEs) (n = 3, 2.8%). One participant (in the "enriched" subgroup) died 114 days after being randomized to tofersen 100 mg. The cause of death was reported as congestive cardiac failure, and the Investigator assessed the event to be unrelated to tofersen.

Table 6: Study 101 Part C participant disposition

	Faster-Progressing Subgroups; mITT		**Slower-Progressing Subgroups; Non-mITT**		**ITT**	
	placebo	**tofersen**	**placebo**	**tofersen**	**placebo**	**tofersen**
Dosed n (%)	21 (100)	39 (100)	15 (100)	33 (100)	36 (100)	72 (100)
Died n (%)	0	1 (3)	0	0	0	1 (1)
Completed study treatment n (%)	19 (91)	33 (85)	14 (93)	30 (91)	33 (92)	63 (88)
Withdrew from study n (%)	2 (10)	6 (15)	1 (7)	2 (6)	3 (8)	8 (11)
- AE	0	1 (3)	0	1 (3)	0	2 (3)
- Consent withdrawn	0	1 (3)	1 (7)	1 (3)	1 (3)	2 (3)
- Death	0	1 (3)	0	0	0	1 (1)
- Disease progression	2 (10)	3 (8)	0	0	2 (6)	3 (4)

- Raw Data Pilot Project analyses and presentation

Table 7: Reasons for study discontinuation in 101 Part C. Disregarding discontinuation in 101 Part C if the subject entered OLE.

		Treatment group	
Visit	**Reason**	**Tofersen 100 mg (n=72)**	**Placebo (n=36)**
DAY57 PART C	PROGRESSIVE DISEASE	1	.
DAY113 PART C	ADVERSE EVENT	1	.
	PROGRESSIVE DISEASE	1	1
	DEATH	1	.
DAY141 PART C	PROGRESSIVE DISEASE	.	1
DAY169 PART C	WITHDRAWAL BY SUBJECT	1	.
END OF STUDY/DAY197 PART C	PROGRESSIVE DISEASE	1	.
	WITHDRAWAL BY SUBJECT	1	1
SAFETY FOLLOW-UP DAY225 PART C	ADVERSE EVENT	1	.

Eight patients in the tofersen group and 3 in the placebo discontinued study 101 Part C. However, due to the 2:1 randomisation, approximately the same percentage of patients completed the study treatment with 92% and 88% for placebo and tofersen, respectively. One death in the tofersen group was considered unrelated to tofersen treatment. Analysis of the discontinuations did not reveal any particular issues of concern. Notably, although patients were intended to be followed after treatment discontinuation, all but one patient who discontinued treatment withdrew from the study.

- **Outcomes and estimation**

ALSFRS-R

Change in ALSFRS-R total score over 28 weeks was assessed in the mITT population as the primary endpoint using JRT in conjunction with MI for withdrawals other than death (Table 8). In this subgroup, a non-statistically significant difference of 1.2 favoring tofersen was observed in ALSFRS-R change from baseline at Week 28 (95% CI, –3.2 to 5.5; p = 0.97 via JRT+MI). The results from the ANCOVA + MI sensitivity analysis support the results from the JRT + MI (nominal p = 0.60). In the non-mITT population, formal statistical testing was not performed as clear separation between treatment arms was not expected in the non-mITT population at 6 months. There were no deaths in this population, negating the need for a JRT. In this population, the treatment difference favoured tofersen (adjusted mean difference [95% CI]: 1.4 [-1.1, 3.9]; nominal p = 0.27 ANCOVA+MI).

Based on the postulation that NfL has a prognostic value, which is not fully established yet, the applicant performed several analyses. *Post hoc* analyses performed in the overall ITT population, adjusting for baseline NfL as a covariate rather than disease duration and including it also as part of the imputation model, show a 2.1-point treatment difference in favor of tofersen (~34% slowing of decline for tofersen relative to placebo) with greater statistical differentiation (95% CI: -0.33, 4.54; JRT+MI nominal p = 0.5015; ANCOVA+MI nominal p = 0.0904), but still non-significant. For performing the subgroup analysis, the applicant has chosen the median baseline value of 75.6 pg/ml NfL. During the Raw Data Pilot Project, the mean NfL concentration at baseline in the ITT population was found to be 100.39 pg/ml and 89.65 pg/ml in the tofersen group and the placebo tofersen group, respectively, indicating an imbalance, which did not favour the tofersen group.

In the faster-progressing NfL subgroup (baseline plasma NfL ≥ median), a 3.9-point treatment difference (95% CI: -1.0 to 8.86; p = 0.1184 via ANCOVA + MI analysis) also did not show statistically significant results in ALSFRS-R change from baseline at Week 28. A favoring trend for tofersen was observed. In the slower-progressing NfL subgroup (baseline plasma NfL < median), a modest trend favoring tofersen was observed in change from baseline at Week 28 (treatment difference of 0.6 points; [95% CI: -1.33, 2.58]).

Due to various reasons, such as drug interruptions and inability to attend clinic visits, some participants missed doses of study treatment; two or more consecutive missed doses may have a potential impact on efficacy. *Post hoc* sensitivity analyses performed in the ITT population show the influence of i) missing at least 2 consecutive doses and ii) outliers resulting from missing at least 2 consecutive missed doses in a small study.

In the view of the applicant, both analyses showed greater treatment differences in favor of tofersen compared to the planned analysis: 2.2 [95% CI: -0.23, 4.54] and 2.4 [95% CI: 0.15, 4.66], demonstrating the influence one or two outliers, due to consecutive missed dosing, can have in a small sample size. The *post hoc* alternative joint rank methodology is presented initially for the mITT population adjusting for baseline disease duration since symptom onset as in the original primary efficacy analysis. The nominal p-value from the alternative JRT is 0.61, which is more consistent with the result from the ANCOVA+MI analysis. When further accounting for the prognostic value of baseline NfL, by replacing baseline disease duration since symptom onset with baseline plasma NfL as a covariate in the model for the mITT population, the p-value becomes smaller in line with other analyses conducted when adjusting for baseline plasma NfL (nominal p = 0.27). The alternative joint rank analysis for the ITT population when adjusting for baseline plasma NfL also gives a more consistent p-value (nominal p-value = 0.06) with the corresponding ANCOVA+MI analysis.

According to the applicant, plasma NfL at baseline turns out to be a better prognostic factor and thus, it has replaced both baseline ALSFRS-R score and disease duration in the ANCOVA models to account for disease progression. Original scale of NfL instead of the logarithm of the NfL value was used due to its better interpretability as a predictor.

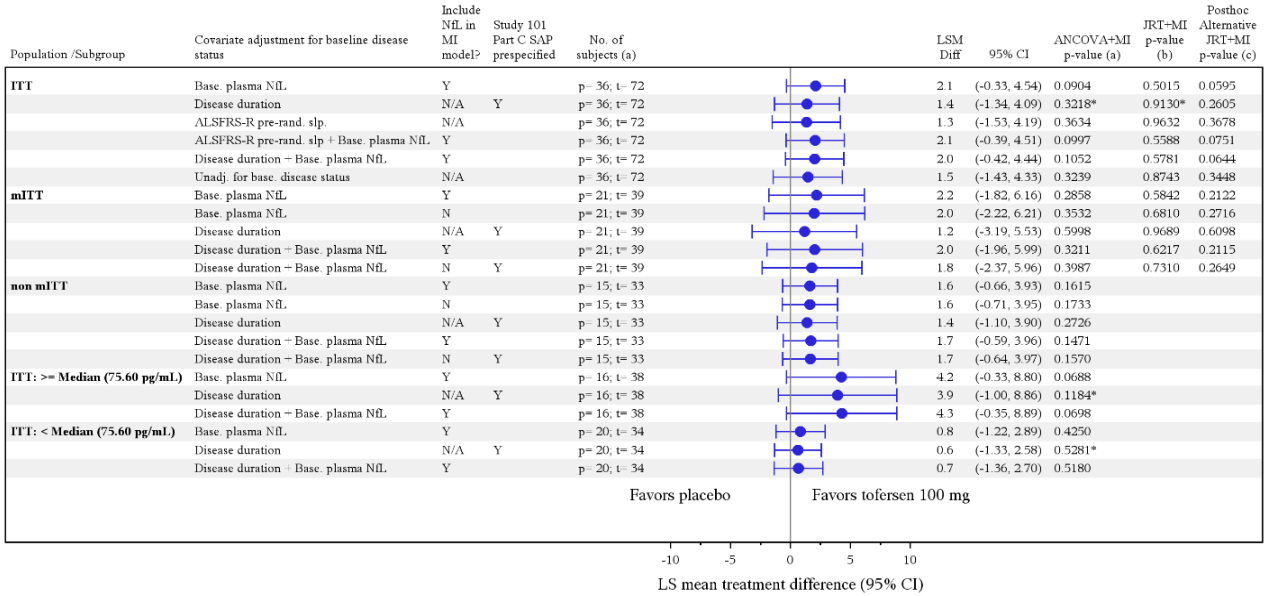
Table 8: Summary of key analyses for ALSFRS-R total score at week 28

		Study 101 Part C	
		Placebo	**Tofersen**
Faster-progressing subgroup	**mITT**		
	N	21	39
	Adjusted mean	-8.14	-6.98
	Tof-plac: Adj. mean difference (95% CI)		**1.2 (-3.2, 5.5)**
	p-value (Joint rank+MI)		0.9689
	Nominal p–value (ANCOVA+MI)		0.5998
	Post hoc (alternative Joint Rank + MI):		
	Nominal p-value adjusting for disease duration Nominal p-value		0.6098
	adjusting for baseline plasma NfL		0.2716
	≥median (75.6 pg/mL) baseline plasma NfL		
	N	16	38
	Adjusted mean	-11.3	-7.3
	Tof-plac: Adj. mean difference (95% CI)		**3.9 (-1.0, 8.9)**
	Nominal p-value ANCOVA+MI		0.1184
Slower-progressing subgroup	**non-mITT**		
	N	15	33
	Adjusted mean	-2.73	-1.33
	Tof-plac: Adj. mean difference (95% CI)		**1.4 (-1.1, 3.9)**
	Nominal p–value (ANCOVA+MI)		0.2726
	<median (75.6 pg/mL) baseline plasma NfL		
	N	20	34
	Adjusted mean	-1.8	-1.2
	Tof-plac: Adj. mean difference (95% CI)		**0.6 (-1.3, 2.6)**
	Nominal p-value ANCOVA+MI		0.5281
ITT population	**Prespecified analyses with disease duration as a covariate**		
	N	36	72
	Adjusted mean	-5.8	-4.5
	Tof-plac: Adj. mean difference (95% CI)		**1.4 (-1.3, 4.1)**
	Post hoc nominal p-value (joint rank + MI)		0.9130
	Post hoc nominal p-value ANCOVA + MI		0.3218
	Post hoc analyses with baseline plasma NfL as a covariate		
	N	36	72
	Adjusted mean	-6.2	-4.1
	Tof-plac: Adj. mean difference (95% CI)		**2.1 (-0.3, 4.5)**
	Nominal p-value (joint rank + MI)		0.5015
	Nominal p-value (ANCOVA + MI)		**0.0904**
	Post hoc (alternative Joint Rank + MI):		
	Nominal p-value adjusting for baseline plasma NfL		0.0595
	Post hoc analyses accounting for ≥2 consecutive missed doses and inclusion of baseline plasma NfL		
	N	36	72
	Adjusted mean	-6.1	-4.0
	Tof - plac: Adj. mean difference (95% CI)		**2.2 (-0.23, 4.54)**
	Nominal p-value (Joint-rank + MI)		0.5656
	Nominal p-value (ANCOVA + MI)		0.0769
	Post hoc analyses excluding outliers and inclusion of baseline plasma NfL		
	N	36	71
	Adjusted mean	-6.2	-3.8
	Tof - plac: Adj. mean difference (95% CI)		**2.4 (0.15, 4.66)**
	Nominal p-value (Joint-rank + MI)		0.4359
	Nominal p-value (ANCOVA + MI)		0.0369

NOTE 1: Lower scores depict a worsening in function. Data are presented at Week 28. Analyses for subgroups defined by median plasma NfL and run-in slope are based on the ITT population. All p-values for the ITT population are post hoc.
NOTE 2: MI was used for missing data. Prespecified model included treatment, use of riluzole or edaravone, relevant baseline score and postbaseline values. Separate models for mITT and non-mITT were used and combined for ITT analyses. In the post hoc analyses adjusting for baseline plasma NfL, this was also included in the MI model.
NOTE 3: Adjusted means, treatment differences and corresponding 95% CIs and nominal p-values for prespecified analyses were obtained from the ANCOVA model for change from baseline in conjunction with MI. Joint-rank p values were obtained from the ANCOVA model for ranked scores where deaths were ranked the lowest, in conjunction with MI for handling missing data due to withdrawals other than death. The original joint rank method is based on the ANCOVA for ranked score for the change from baseline and includes

original scale values for continuous covariates; the alternative joint rank method is based on the ANCOVA for change of ranked score pre- and post-treatment and includes ranked covariates for continuous variables. The ANCOVA models included treatment as a fixed effect and adjusted for the following covariates: baseline disease duration since symptom onset, relevant baseline score, and use of riluzole or edaravone. For analyses adjusting for baseline plasma NfL, this is included in the model instead of baseline disease duration.

Figure 10: 233AS101 Part C: Forest plot of ALSFRS-R total score change from baseline to week 28



Population /Subgroup	Covariate adjustment for baseline disease status	Include NfL in MI model?	Study 101 Part C SAP prespecified	No. of subjects (a)	LSM Diff	95% CI	ANCOVA+MI p-value (a)	JRT+MI p-value (b)	Posthoc Alternative JRT+MI p-value (c)
ITT	Base. plasma NfL	Y		p= 36; t= 72	2.1	(-0.33, 4.54)	0.0904	0.5015	0.0595
	Disease duration	N/A	Y	p= 36; t= 72	1.4	(-1.34, 4.09)	0.3218*	0.9130*	0.2605
	ALSFRS-R pre-rand. slp.	N/A		p= 36; t= 72	1.3	(-1.53, 4.19)	0.3634	0.9632	0.3678
	ALSFRS-R pre-rand. slp + Base. plasma NfL	Y		p= 36; t= 72	2.1	(-0.39, 4.51)	0.0997	0.5588	0.0751
	Disease duration + Base. plasma NfL	Y		p= 36; t= 72	2.0	(-0.42, 4.44)	0.1052	0.5781	0.0644
	Unadj. for base. disease status	N/A		p= 36; t= 72	1.5	(-1.43, 4.33)	0.3239	0.8743	0.3448
mITT	Base. plasma NfL	Y		p= 21; t= 39	2.2	(-1.82, 6.16)	0.2858	0.5842	0.2122
	Base. plasma NfL	N		p= 21; t= 39	2.0	(-2.22, 6.21)	0.3532	0.6810	0.2716
	Disease duration	N/A	Y	p= 21; t= 39	1.2	(-3.19, 5.53)	0.5998	0.9689	0.6098
	Disease duration + Base. plasma NfL	Y		p= 21; t= 39	2.0	(-1.96, 5.99)	0.3211	0.6217	0.2115
	Disease duration + Base. plasma NfL	N	Y	p= 21; t= 39	1.8	(-2.37, 5.96)	0.3987	0.7310	0.2649
non mITT	Base. plasma NfL	Y		p= 15; t= 33	1.6	(-0.66, 3.93)	0.1615		
	Base. plasma NfL	N		p= 15; t= 33	1.6	(-0.71, 3.95)	0.1733		
	Disease duration	N/A	Y	p= 15; t= 33	1.4	(-1.10, 3.90)	0.2726		
	Disease duration + Base. plasma NfL	Y		p= 15; t= 33	1.7	(-0.59, 3.96)	0.1471		
	Disease duration + Base. plasma NfL	N	Y	p= 15; t= 33	1.7	(-0.64, 3.97)	0.1570		
ITT: >= Median (75.60 pg/mL)	Base. plasma NfL	Y		p= 16; t= 38	4.2	(-0.33, 8.80)	0.0688		
	Disease duration	N/A	Y	p= 16; t= 38	3.9	(-1.00, 8.86)	0.1184*		
	Disease duration + Base. plasma NfL	Y		p= 16; t= 38	4.3	(-0.35, 8.89)	0.0698		
ITT: < Median (75.60 pg/mL)	Base. plasma NfL	Y		p= 20; t= 34	0.8	(-1.22, 2.89)	0.4250		
	Disease duration	N/A	Y	p= 20; t= 34	0.6	(-1.33, 2.58)	0.5281*		
	Disease duration + Base. plasma NfL	Y		p= 20; t= 34	0.7	(-1.36, 2.70)	0.5180		

NOTE 1: SAP V2.0 is the integrated efficacy SAP that was finalized prior to the final database lock for 233AS101 and interim lock for 233AS102 based on 16 July 2021 data cut; prespecified analyses were primarily based on disease progression subgroups and adjusted for disease duration since symptom onset. SAP V3.0 was an amendment to the integrated efficacy SAP following the initial data readout and incorporated plasma NfL as a covariate with the focus on the ITT population. SAP V3.0 was finalized prior the interim lock for 233AS102 based on 16 January 2022 data cut.
NOTE 2: For ITT population, multiple imputation including the specified covariate(s), use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include the specified covariate(s), baseline ALSFRS-R, and use of riluzole or edaravone.
NOTE 3: For the analyses including disease duration since symptom onset in mITT and non mITT population, multiple imputation including use of riluzole or edaravone and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include covariates for baseline disease duration since symptom onset, baseline ALSFRS-R and use of riluzole or edaravone. For the other analyses in mITT and non mITT population, multiple imputation including baseline plasma NfL, use of riluzole or edaravone and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include covariates for the specified covariate(s), baseline plasma NfL, baseline ALSFRS-R and use of riluzole or edaravone.
* No statistical testing was prespecified in SAP Version 2; all p-values are post hoc for SAP V2 analyses.
(a) From the listed ANCOVA analysis based on change from baseline.
(b) From the ANCOVA on ranked scores; deaths are ranked the lowest; MI is used for handling missing data due to withdrawals other than death.
(c) From the ANCOVA on change of ranked scores pre- and post-treatment; deaths are ranked the lowest; MI is used for handling missing data due to withdrawals other than death.
Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square; P+DS = placebo + delayed-start tofersen 100 mg; ES = Early-start tofersen 100 mg.

From the Forest plot above it can be observed that various analyses ALSFRS-R total score change from baseline to week 28 show a favouring trend for tofersen, without, however, reaching statistical significance.

The most important issue is the outcome of the various analyses with the ALSFRS-R scale. The results in primary analysis of the functional scale ALSFRS-R were not statistically significant in the pre-specified mITT population. Hence, the study has failed the pre-specified conditions. For the general ALS population functional decline averages about 1 point per month in untreated patients, however the decline might not be linear. The difference of 2.1 points of ALSFRS-R total score change from baseline to week 28 in the ITT population using *post hoc* analysis with baseline plasma NfL as a covariate between tofersen and placebo is small, and the clinical relevance cannot be determined. This, as acknowledged by the applicant, can be due to study design (e.g., enrichment strategy and study duration) as well as inherent heterogeneity in SOD1-ALS.

McElhiney et al in 2014 reported that while the ALSFRS-R is widely used in clinical trials, its relationship to clinically meaningful changes is elusive and may require a longer time-frame than the 6 months period utilized in their study. During their six-month study, ALSFRS-R total scores declined on average 5.7 points (SD=4.7). This is close to the mean 0.8-point drop/month expected, which for six months would be a 4.8-point decline (McElhiney et al 2014).

In a more recent publication, the mean decline per month was −2.03 points per month on the normed ROADS score (SD = 2.44) and −0.76 points per month on the ALSFRS-R total sum score (SD = 1.09) (Fournier et al 2022). On the ALSFRS-R, the mean decline in sum-score was −0.64 points (SD = 2.17) for patients who rated themselves as better or unchanged, −3.88 points (SD 3.98) for patients who rated themselves as a little worse, and −7.21 points (SD = 6.08) for participants who rated themselves as much worse. Based on the difference between mean change in score for participants who rated themselves as a little worse compared to unchanged/better, the Minimal important difference (MID) was set to 5.81 (3.98%) for ROADS (based on the normed score, total possible points = 146) and MID to 3.24 (6.75%) for ALSFRS-R (based on 48 possible points). The authors concluded that changes that are on average less than 5.81 points (3.98%) on the normed ROADS score or less than 3.24 points (6.75%) on the ALSFRS-R sum-score over an average period of 6.41 months may not be clinically meaningful according to a patient-defined approach (Fournier et al 2022).

SVC

The treatment difference favoured the tofersen group compared to the placebo group for both the mITT population (treatment difference of 7.9 in percent predicted SVC change from baseline at Week 28 [95% CI: -3.5, 19.3]; nominal p = 0.323 [JRT+MI]) and the non-mITT population (treatment differences of 4.6 [95% CI: −1.2, 10.5]).

In the faster-progressing NfL subgroup (baseline plasma NfL ≥ median), a treatment difference of 9.91 percent predicted SVC change from baseline at Week 28 favoring tofersen was observed (95% CI: -2.27, 22.09). In the slower-progressing NfL subgroup (baseline plasma NfL < median), a 6.05 percent predicted treatment difference favoring tofersen was observed in percent predicted SVC change from baseline at Week 28 (95% CI: -0.58, 12.68).

Post hoc analyses performed in the overall ITT population, adjusting for baseline NfL as a covariate rather than disease duration and baseline ALSFRS-R score, show an 8.5 percent-predicted treatment difference at Week 28 in favor of tofersen (~54% slowing of decline for tofersen relative to placebo; [95% CI: 1.8, 15.2]; JRT+MI nominal p = 0.0689; ANCOVA+MI nominal p = 0.0128).

It is noted that as in the case of ALSFRS-R total score change from baseline to week 28, no statistically significant difference was observed on the analysis of SVC in the primary analysis mITT population. The *post hoc* analysis via JRT+MI was also statistically non-significant. Furthermore, only a very small difference (<8.5%) in the percent predicted SVC was observed when a post hoc analyses with baseline plasma NfL as a covariate was performed in the ITT population.

Table 9: Summary of key analyses for percent predicted SVC at week 28

		Study 101 Part C	
		Placebo	**Tofersen**
Faster-progressing subgroup	**mITT** N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p-value (Joint rank+MI) Nominal p–value (ANCOVA+MI)	 21 -22.2	 39 -14.31 **7.9 (-3.5, 19.3)** **0.3233** 0.1755
	≥ median (75.6 pg/mL) baseline plasma NfL N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p-value ANCOVA+MI	 16 -26.1	 38 -16.2 **9.9** (-2.3, 22.1) 0.1108
Slower-progressing subgroup	**non-mITT** N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p–value (ANCOVA+MI)	 15 -4.9	 33 -0.3 4.6 (-1.2, 10.5) 0.1210
	< median (75.6 pg/mL) baseline plasma NfL N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p-value ANCOVA+MI	 20 -5.6	 34 0.4 **6.1** (-0.6, 12.7) 0.0736
ITT population	**Prespecified analyses with disease duration as a covariate** N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Post hoc nominal p-value (joint rank + MI) Post hoc nominal p-value ANCOVA + MI	 36 -14.8	 72 -7.9 6.9 (-0.1, 13.8) 0.1517 0.0522
	Post hoc analyses with baseline plasma NfL as a covariate N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p-value (joint rank + MI) Nominal p-value (ANCOVA + MI)	 36 -15.8	 72 -7.3 **8.5 (1.8, 15.2)** **0.0689** 0.0128

NOTE 1: Lower values depict a worsening in function. Data are presented at Week 28. Analyses for subgroups defined by median plasma NfL and run-in slope are based on the ITT population. All p-values for the ITT population are post hoc.
NOTE 2: MI was used for missing data. For prespecified analysis, separate imputation models for mITT and non-mITT were used and combined for the ITT analysis. The model included treatment, use of riluzole or edaravone, baseline ALSFRS-R score, relevant baseline score and postbaseline values. For post hoc ITT analyses adjusted for baseline plasma NfL, the multiple imputation model was based on all participants in the ITT population and included treatment, baseline plasma NfL, use of riluzole or edaravone, relevant baseline score and postbaseline values.
NOTE 3: Adjusted means, treatment differences and corresponding 95% CIs and nominal p-values were obtained from the ANCOVA model for change from baseline in conjunction with MI. Joint-rank p-values were obtained from the ANCOVA model for ranked scores where deaths are ranked the lowest, in conjunction with MI for handling missing data due to withdrawals other than death. The ANCOVA models for prespecified analyses included treatment as a fixed effect and adjusted for the following covariates: baseline disease duration since symptom onset, baseline ALSFRS-R score, relevant baseline score, and use of riluzole or edaravone. The ITT analysis adjusted for baseline plasma NfL used the same model except baseline disease duration since symptom onset and baseline ALSFRS-R score were replaced by baseline plasma NfL.

HHD Megascore

Modest trends suggesting slowing of decline in muscle strength were observed with tofersen administration in the mITT and non-mITT populations: differences of 0.02 (95% CI: -0.21, 0.26) and 0.09 (95% CI: -0.08, 0.26) favoring tofersen were observed, respectively. These trends were most apparent in lower limb muscles as compared with upper limb muscles.

In the faster-progressing NfL subgroup (baseline plasma NfL ≥ median), a difference of 0.13 (95% CI: -0.10, 0.37) favoring tofersen was observed in HHD megascore change from baseline at Week 28. In the slower-progressing NfL subgroup (baseline plasma NfL < median), a difference of 0.09 favoring tofersen was observed in change from baseline at Week 28.

Post hoc analyses performed in the overall ITT population, adjusting for baseline NfL as a covariate rather than disease duration, show a difference of 0.10 treatment difference in HHD megascore at Week 28 in favor of tofersen (~31% slowing of decline for tofersen relative to placebo; [95% CI: -0.04, 0.23]; ANCOVA+MI nominal p = 0.1547). The treatment difference in the ITT population for HHD lower extremities was 0.22 in favor of tofersen (95% CI: 0.04, 0.40) with a nominally statistically significant difference (p = 0.0169).

As in the case of ALSFRS-R total score and SVC, no statistically significant difference was observed on the analysis of HDD in the primary analysis mITT population. The *post hoc* analysis via ANCOVA+MI was also statistically non-significant. Furthermore, a very small difference (<0.10) in HDD was also observed when a *post hoc* analyses with baseline plasma NfL as a covariate was performed in the ITT population.

Table 10: Summary of key analyses for HHD megascore at week 28

		Study 101 Part C	
		Placebo	**Tofersen**
Faster-progressing subgroup	**mITT** N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p–value (ANCOVA+MI)	21 -0.37	39 -0.34 **0.02 (-0.21, 0.26)** **0.8390**
	⩾ median (75.6 pg/mL) baseline plasma NfL N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p-value ANCOVA+MI	16 -0.49	38 -0.36 0.13 (-0.10, 0.37) 0.2690
Slower-progressing subgroup	**non-mITT** N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p–value (ANCOVA+MI)	15 -0.18	33 -0.09 0.09 (-0.08, 0.26) 0.2832
	< median (75.6 pg/mL) baseline plasma NfL N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p-value ANCOVA+MI	20 -0.17	34 -0.09 0.09 (-0.10, 0.27) 0.3508
ITT population	**Prespecified analyses with disease duration as a covariate** N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Post hoc nominal p-value ANCOVA + MI	36 -0.29	72 -0.23 0.06 (-0.09, 0.21) 0.4416

		Study 101 Part C	
		Placebo	**Tofersen**
	Post hoc analyses with baseline plasma NfL as a covariate N Adjusted mean Tof-plac: Adj. mean difference (95% CI) Nominal p-value ANCOVA + MI	 36 -0.32	 72 -0.23 **0.10 (-0.04, 0.23)** **0.1547**

NOTE 1: Lower scores depict a worsening in function. Data are presented at Week 28. Analyses for subgroups defined by median plasma NfL and run-in slope are based on the ITT population. All p-values for the ITT population are post hoc.
NOTE 2: MI was used for missing data. For prespecified analysis, separate imputation models for mITT and non-mITT were used and combined for the ITT analysis. The model included treatment, use of riluzole or edaravone, baseline ALSFRS-R score, relevant baseline score and postbaseline values. For post hoc ITT analyses adjusted for baseline plasma NfL, the multiple imputation model was based on all participants in the ITT population and included treatment, baseline plasma NfL, use of riluzole or edaravone, relevant baseline score and postbaseline values.
NOTE 3: Adjusted means, treatment differences and corresponding 95% CIs and nominal p-values were obtained from the ANCOVA model for change from baseline, in conjunction with MI, including treatment as a fixed effect and adjusting for the following covariates: baseline disease duration since symptom onset, relevant baseline score, and use of riluzole or edaravone. The ITT analysis adjusted for baseline plasma NfL used the same model except baseline disease duration since symptom onset was replaced by baseline plasma NfL.

Weight

Weight was collected as part of the safety battery in Study 101 Part C. Weight loss has been found to be a strong independent predictor of survival in ALS. Over time, the average weight increased in the tofersen group (mean change from baseline at Week 28 [±SD]: 0.5 kg [±4.4]) and decreased in the placebo group (mean change from baseline at Week 28 [±SD]: -1.6 kg [±5.4]).

It can be agreed that body weight is an important factor strongly associated with survival in ALS. However, the mean changes from baseline to week 28 are too small to allow any meaningful comparisons. Notably, weight was not an efficacy endpoint according to protocol.

Survival in Study 101 Part C (week 28)

- *Applicant's Analysis*

The applicant recognizing the progressive and fatal nature of SOD1-ALS, time to death or PV and time to death assessed as key secondary endpoints in Study 101 Part C. PV was defined as ≥ 22 hours of mechanical ventilation (invasive or noninvasive) per day for ≥ 21 consecutive days. The threshold for PV of ≥ 21 consecutive days was extended beyond that used in previous studies (e.g., ≥ 7 to 10 days) in an effort to differentiate between PV and potential acute reversible illnesses (e.g., pneumonia) necessitating temporary ventilatory support. The definition is based solely on duration of use and does not distinguish between invasive and noninvasive ventilation. Recognizing that standards of care for tracheostomy vary globally, placement of a tracheostomy tube was not considered an event unless the threshold for ventilatory support was met.

Time to death or permanent ventilation was determined in a blinded fashion by a central, independent endpoint adjudication committee through review of data.

The applicant admitted that while survival is considered the gold standard for demonstrating efficacy, it necessitates studies of adequate size and duration. When designing the study, it was recognized that the short study duration (6-month duration of Study 101 Part C) and inter- and intra-SOD1 mutation heterogeneity in disease duration would make the detection of an effect on survival challenging.

Median time to death or PV and median time to death were not estimable in Study 101 Part C due to the limited number of events observed (CSR 233AS101 Part C).

The applicant has provided an explanation for their choice of ≥ 21 consecutive days of ≥ 22 hours of mechanical ventilation. This number (of ≥ 21 consecutive days) is considered conservative and additional analyses were performed with the Raw Data Pilot Project (please see below).

In the mITT population, a similar proportion of participants in the placebo (2 participants [9.5%] with events of PV) and tofersen (4 participants [10.3%] – 1 death due to congestive heart failure and 3 events of PV) groups experienced events of death or PV. The percentage of patients in the mITT population (and consequently in the ITT population) with an event of death or PV was similar in the placebo (9.5%) and tofersen (10.3%) groups. The numbers are too small to be considered as important survival data.

No events of death or PV occurred in the non-mITT population.

Table 11: Summary of time to death or permanent ventilation - mITT Population 233AS101 Part C: Secondary endpoint analysis: Summary of time to death or permanent ventilation - mITT population

	Placebo (N=21)	**Tofersen (N=39)**
Number of subjects with an event of death or permanent ventilation	2 (9.5)	4 (10.3)
Death	0	1 (2.6)
Permanent ventilation	2 (9.5)	3 (7.7)
Number of days with ventilation use for at least 22 hours per day		
n	**2**	**3**
Mean (SD)	15.0 (18.38)	20.0 (18.52)
Median	15.0	21.0
Q1,Q3	2.0, 28.0	1.0, 38.0
Min, Max	2, 28	1, 38

Patient-Reported Outcome Measures Study 101 Part C

The results of the PRO were not statistically significant but have shown a trend in favour of tofersen.

ALSAQ-5

Analysis of the ALSAQ-5 total scores indicated less worsening from baseline to Week 28 in the tofersen group for both mITT and non-mITT populations: -5.6-point treatment difference (95% CI: -15.55, 4.37) and -1.6-point treatment difference (95% CI: -9.55, 6.29), respectively.

In the *post hoc* ITT population analysis where baseline NfL was adjusted, a -5.7-point treatment difference favoring tofersen was observed in ALSAQ-5 total score change from baseline at Week 28 (95% CI: -11.83, 0.40; nominal p = 0.0668).

FSS

In the mITT population, a trend favoring tofersen was observed in FSS change from baseline at Week 28, suggesting less fatigue (-4.91-point treatment difference [95% CI: -11.23, 1.42]). This trend was not evident in the non-mITT population (2.84-point treatment difference [95% CI: -4.72, 10.40])

In the post hoc ITT population analysis where baseline NfL was adjusted, a -2.4-point treatment difference favoring tofersen was observed in FSS total score change from baseline at Week 28 (95% CI: -7.45, 2.55; nominal p = 0.3365).

EQ-5D-5L

In the mITT population, a smaller mean change from baseline to Week 28 in the EQ-5D-5L utility score in the tofersen group (-0.16) was observed compared with placebo group (-0.35), with a 0.20-point

treatment difference in change from baseline at Week 28 (95% CI: 0.062, 0.332; nominal p = 0.0043). This trend was not evident in the non-mITT population as both treatment groups remained stable. In the post hoc ITT population analysis where baseline NfL was adjusted, tofersen administration was also associated with less decline as assessed by EQ-5D-5L utility score and VAS score change from baseline at Week 28.

- **Ancillary analyses (Study 101 Part C (week 28 and week 52)**

For the ITT population of study 101 Part C, analyses were performed with the prespecified disease progression subgroups mITT (N=60) (also referred to as the enriched or faster progressing/faster progressor subgroup) vs. non-mITT (n = 48) (also referred to as the other or slower progressing/slower progressor subgroup) as well as subgroups above and below the median NfL.

Applicant's analyses

The applicant has performed several analyses (secondary, sensitivity and supplementary) of the ALSFRS-R total score, as it was presented above for the ALSFRS-R section.

Raw Data Pilot Project Analyses

Under the Raw Data Pilot Project, the following analyses were performed. Comparisons between allocated treatment groups in 101 Part C, at week 28 and week 52, i.e. extending into 102 OLE, using reference-based imputations on missing values after intercurrent events, are shown in the two tables below.

The following reference-based imputation rules have been applied:

- Jump-to-reference (J2R): Missing values in the early start tofersen treatment group following an intercurrent event will be imputed using the distribution of the delayed start tofersen treatment group (i.e. the reference), except deaths in which case missing values will be imputed as zero for both treatment groups.
- Copy-reference (CR): Subject with missing values following intercurrent events will have entire series imputed based on reference group, except deaths in which case missing values will be imputed as zero for both treatment groups.
- Copy-increments-from-reference (CIR): Missing values in the early start tofersen treatment group following a intercurrent event will be imputed following a parallel track to the reference group, except deaths in which case missing values will be imputed as zero for both treatment groups.

The following events are regarded as intercurrent events with impact on the imputation applied. The list includes all causes for missing values following a monotone pattern, i.e. any missing values after last recording. Patterns consisting of intermittent missing values are imputed as occurring at random (MAR), and thus imputed based on a distribution based on allocated treatment group.

List of intercurrent events:

- Treatment discontinuation
- PV assistance
- Death
- Loss to follow-up (which is not an intercurrent event in itself but assumed to be preceded by an intercurrent event such as treatment discontinuation)

In case of more than one intercurrent for a subject, any missing values following the first will be imputed. The rule for imputation in case of deaths overrules the rule of any preceding intercurrent event.
During the planning of the trial treatment interruptions of more than 2 consecutive doses were also regarded as an intercurrent event. However, all subjects experienced treatment interruption of four consecutive doses, or more, and thus this was excluded from the list.

Table 12: ALSFRS-R change from baseline sensitivity analyses: imputation with CIR, CR & J2R and analyses with applicants MMRM model at week 28 (time=197) and week 52 (time=365) (raw data pilot project)

Imputation	Time point	Statistics	Treatment	Estimate	SE	Lower 95% CL	Upper 95% CL	DF	Prob < t
CIR	197	Difference	(NA)	**-1.00**	1.679	-4.36	2.35	60.3	0.5522
		LS Mean	Delayed start tofersen 100 mg	-6.10	1.412	-8.92	-3.28	.	.
		LS Mean	Early start tofersen 100 mg	-5.10	0.953	-7.00	-3.19	.	.
	365	Difference	(NA)	**-1.59**	2.449	-6.48	3.31	60.0	0.5199
		LS Mean	Delayed start tofersen 100 mg	-8.98	2.073	-13.13	-4.84	.	.
		LS Mean	Early start tofersen 100 mg	-7.40	1.412	-10.22	-4.57	.	.
CR	197	Difference	(NA)	**-0.98**	1.681	-4.34	2.39	60.5	0.5634
		LS Mean	Delayed start tofersen 100 mg	-6.10	1.412	-8.93	-3.28	.	.
		LS Mean	Early start tofersen 100 mg	-5.13	0.953	-7.03	-3.22	.	.
	365	Difference	(NA)	**-1.53**	2.455	-6.45	3.38	60.0	0.5342
		LS Mean	Delayed start tofersen 100 mg	-8.99	2.072	-13.14	-4.85	.	.
		LS Mean	Early start tofersen 100 mg	-7.46	1.408	-10.27	-4.64	.	.
J2R	197	Difference	(NA)	**-0.97**	1.684	-4.33	2.40	60.7	0.5686
		LS Mean	Delayed start tofersen 100 mg	-6.11	1.413	-8.93	-3.28	.	.
		LS Mean	Early start tofersen 100 mg	-5.14	0.968	-7.08	-3.21	.	.
	365	Difference	(NA)	**-1.43**	2.465	-6.36	3.50	60.7	0.5636
		LS Mean	Delayed start tofersen 100 mg	-9.01	2.076	-13.16	-4.86	.	.
		LS Mean	Early start tofersen 100 mg	-7.58	1.464	-10.50	-4.65	.	.

ALSFRS-R: ALS Functional Rating Scale – Revised; CIR: copy increment in reference; CR: copy reference; ITT: intent-to-treat; J2R: jump to reference

It is noted that the calculations were performed using macros with SAS.

Table 13: ALSFRS-R change from baseline sensitivity analyses: imputations with CIR, CR & J2R and analyses with applicants MMRM model + baseline NFL covariate at week 28 (time=197) and week 52 (time=365) (raw data pilot project)

Imputation	Time point	Statistics	Treatment	Estimate	SE	Lower 95% CL	Upper 95% CL	DF	Prob < t
CIR	197	Difference	(NA)	**-1.70**	1.549	-4.79	1.39	68.8	0.2749
		LS Mean	Delayed start tofersen 100 mg	-6.57	1.194	-8.95	-4.19	.	.
		LS Mean	Early start tofersen 100 mg	-4.87	1.012	-6.89	-2.85	.	.
	365	Difference	(NA)	**-2.85**	2.178	-7.20	1.50	68.0	0.1950
		LS Mean	Delayed start tofersen 100 mg	-9.83	1.696	-13.21	-6.44	.	.
		LS Mean	Early start tofersen 100 mg	-6.98	1.440	-9.85	-4.10	.	.
CR	197	Difference	(NA)	**-1.68**	1.547	-4.76	1.41	69.0	0.2825
		LS Mean	Delayed start tofersen 100 mg	-6.58	1.194	-8.96	-4.19	.	.

Imputation	Time point	Statistics	Treatment	Estimate	SE	Lower 95% CL	Upper 95% CL	DF	Prob < t
		LS Mean	Early start tofersen 100 mg	-4.90	1.010	-6.92	-2.88	.	.
	365	Difference	(NA)	**-2.80**	2.175	-7.14	1.54	68.4	0.2025
		LS Mean	Delayed start tofersen 100 mg	-9.83	1.695	-13.22	-6.45	.	.
		LS Mean	Early start tofersen 100 mg	-7.04	1.431	-9.89	-4.18	.	.
J2R	197	Difference	(NA)	**-1.68**	1.555	-4.78	1.42	68.7	0.2837
		LS Mean	Delayed start tofersen 100 mg	-6.58	1.194	-8.96	-4.19	.	.
		LS Mean	Early start tofersen 100 mg	-4.90	1.024	-6.94	-2.85	.	.
	365	Difference	(NA)	**-2.73**	2.203	-7.13	1.66	67.9	0.2192
		LS Mean	Delayed start tofersen 100 mg	-9.85	1.697	-13.23	-6.46	.	.
		LS Mean	Early start tofersen 100 mg	-7.11	1.492	-10.09	-4.14	.	.

ALSFRS-R: ALS Functional Rating Scale – Revised; CIR: copy increment in reference; CR: copy reference; ITT: intent-to-treat; J2R: jump to reference

The replication from the applicant of these analyses showed discrepancies to the above results that were not of a magnitude to impact the overall interpretation of the results. In additional analyses, the applicant added interaction terms between baseline plasma NfL and riluzole/ edaravone use by visit in the imputation models (in addition to ALSFRS-R total score at each visit, baseline plasma NfL, and riluzole/edaravone use), which were used for generating the results presented and reported by the applicant (Table 14).

Table 14: Reference-based imputation on missing data after intercurrent event – ALSFRS-R total score change from baseline at week 28 and week 52 – ITT population

Macro	Timepoint	Imputation	Estimated Difference (95% Confidence Interval)	p-value
Five Macros	Week 28	MAR	2.0 (-0.54, 4.58)	0.1222
		J2R	1.7 (-0.96, 4.38)	0.2089
		CR	1.8 (-0.89, 4.40)	0.1943
		CIR	1.8 (-0.85, 4.42)	0.1847
	Week 52	MAR	3.7 (0.32, 7.03)	0.0319
		J2R	3.0 (-0.59, 6.60)	0.1012
		CR	3.1 (-0.38, 6.61)	0.0806
		CIR	3.2 (-0.26, 6.71)	0.0699
RMConjPlus	Week 28	MAR	2.1 (-0.44, 4.66)	0.1087
		J2R	1.9 (-0.71, 4.49)	0.1566
		CR	1.9 (-0.63, 4.52)	0.1419
		CIR	2.0 (-0.61, 4.53)	0.1378
	Week 52	MAR	3.7 (0.42, 6.97)	0.0294
		J2R	3.1 (-0.35, 6.47)	0.0815
		CR	3.3 (-0.01, 6.61)	0.0533

Macro	Timepoint	Imputation	Estimated Difference (95% Confidence Interval)	p-value
		CIR	3.3 (0.00, 6.62)	0.0531

ALSFRS-R: ALS Functional Rating Scale – Revised; CIR: copy increment in reference; CR: copy reference; ITT: intent-to-treat; J2R: jump to reference; MAR: missing at random.

2.6.5.2.2. Study 102 LTE (on-going)

Overview of Methods, Participants and Treatment

This study is an ongoing multicentre, OLE study to assess the long-term safety, tolerability, PK, and effect on disease progression of tofersen/BIIB067 administered to previously treated adults with ALS caused by SOD_1 mutation.

Prespecified interim cuts of the ongoing Study 102 data were conducted on 16 July 2021, 16 January 2022 and the latest one was 28 February 2023 for the efficacy. An additional data cut for safety was performed on 15 July 2022.

Tofersen is administered by IT injection to participants with SOD1-ALS who completed Part A, B, or C of Study 233AS101. Of the 159 eligible participants who completed Study 101, a total of 139 participants enrolled from Study 101 Parts A, B, and C: 44 participants enrolled from Study 101 Parts A and B and 95 participants enrolled from Part C.

Participants in Study 233AS101 Parts A + B must have had a washout ≥ 16 weeks between the last dose of study treatment received in Study 233AS101 and the first dose of tofersen received in the current Study 233AS102. Participants in Part C did not require a washout period.

Participants initially received 3 loading doses of tofersen 20 mg (Group 1), 40 mg (Group 2), or 60 mg (Group 3) approximately 2 weeks apart, and 10 maintenance doses, approximately monthly, by IT injection.

It is noted that CSF NfL data were available for the first time during the January 2022 interim analysis. These data were presented to Week 24 in both subgroups and the ITT population due to sparse data beyond that time-point.

Limitations of study 102 LTE

There are some limitations in the analyses of Study 102 alone and some trends may appear to differ slightly from the overall trends observed in the integrated analyses of Study 101 Part C and Study 102 comparing early-start with placebo/delayed-start participants. These are due to various reasons outlined below.

The baseline mean values in Study 102 are not equivalent to the Week 28 observed mean values in Study 101 Part C or the integrated analysis due to the following reasons:

- A subset of participants enrolled from Study 101 Part C into Study 102 (95 of 108 participants), so the baseline mean values for Study 102 are not the same as the Week 28 mean values observed in Study 101 Part C.
- Some participants were not able to attend a clinic visit at Week 28 in Study 101 Part C and have missing assessments for SVC and HHD but may have enrolled later into Study 102; these participants do not have observed data for the Week 28 assessment in Study 101 Part C but have baseline values for Study 102
- If baseline value was not available for Study 102, the screening value was used.

Other factors contributing to these differences include the level of missing assessments, particularly for SVC, and the adjustment for baseline NfL in integrated analyses, which is not feasible to account for in Study 102 analyses.

Summary of results of study 102 LTE

As of the 16 January 2022 interim data cut, in participants who received placebo in Study 233AS101 Part C and initiated tofersen 100 mg in Study 233AS102, initiation of treatment with tofersen 100 mg produced a robust biological effect, as measured by reduction of total CSF SOD1 protein, plasma and CSF NfL, and plasma and CSF pNfH, even when started 6 months later in disease. According to the applicant, clinical data from participants from Study 233AS101 Part C demonstrated that participants who had previously received tofersen 100 mg in Study 233AS101 Part C started Study 233AS102 with better clinical function (as measured by baseline ALSFRS-R total score, percent predicted SVC, and HHD megascore values) than did participants who had previously received placebo. During 24 weeks of open-label treatment with tofersen, participants had modest declines (as measured by ALSFRS-R total score and percent predicted SVC) or maintenance (as measured by HHD megascore) of clinical function, with changes of similar magnitude over time regardless of previous treatment group.

Participants who were originally enrolled in Study 233AS101 Parts A and B are a unique population of participants who received either placebo or tofersen (in doses ranging from 20 to 100 mg) in Study 233AS101, then had a ≥ 16-week washout of study medication before initiating tofersen at varying dose levels in Study 233AS102. In those participants that ultimately received tofersen 100 mg in Study 233AS102, reductions in CSF SOD1 protein levels, plasma pNfH, and CSF pNfH were observed and sustained over 196 weeks. According to the applicant, tofersen treatment also resulted in a sustained clinical effect, with participants experiencing small declines in respiratory function (measured by percent predicted SVC) and maintenance of strength and clinical function (as measured by HHD megascore and ALSFRSR total scores) over 196 weeks of open-label treatment in Study 233AS102.

The data from the study 102 LTE alone are from an open-label design and as such, they do not have the convincing value of controlled data. However, the pooling of data from study 101 Part C and 102 is worth discussing due to the long-term treatment of SOD1-ALS patients.

2.6.5.3. Biomarkers

2.6.5.3.1. Biomarkers results only for Study 101 Part C (week 28)

Total CSF SOD1 Protein - Change from Study 101 Part C Baseline to Week 28 (Day 197)

In the mITT population, a reduction in total CSF SOD1 protein was observed at Week 28 in the tofersen group compared to the placebo group (difference in geometric mean ratios for tofersen to placebo of 38% and nominal p <0.0001).

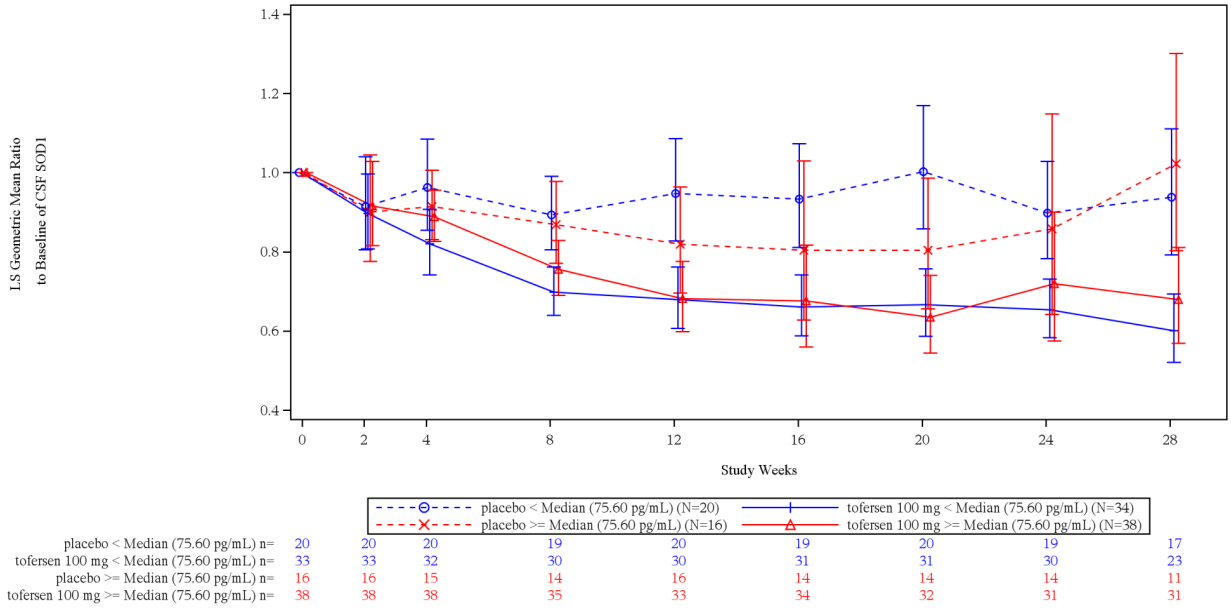
In the non-mITT population, a reduction in total CSF SOD1 protein was also observed at Week 28 in the tofersen group compared to the placebo group (difference in geometric mean ratios for tofersen to placebo of 26% and nominal p = 0.0007).

In the ITT population, a reduction in total CSF SOD1 protein was also observed at Week 28 in the tofersen group compared to the placebo group (difference in geometric mean ratios for tofersen to placebo of 34% and post-hoc nominal p < 0.0001).

Tofersen is designed to bind and degrade SOD1 mRNA to reduce SOD1 protein synthesis and accumulation via RNase-H-dependent degradation of SOD1 mRNA. All SOD1 variants associated with ALS > 200 identified to date are assumed to cause SOD1 gain of function, i.e., they lead to an

accumulation of mutant SOD1 protein that is believed to underlie the pathogenicity of SOD1 ALS. According to the applicant, although a target therapeutic threshold for total CSF SOD1 reduction has not been established, reductions of at least 20%, a threshold beyond the assay and biologic variabilities, was aimed as confirmation of target engagement.

Figure 11: Line Plot of Total CSF SOD1 Protein Adjusted Geometric Mean Ratio to Baseline Values (95% CI) by Visit from ANCOVA Analysis Using MI by Baseline Plasma NfL Level (Median) – ITT Population 233AS101 Part C: Secondary endpoint subgroup analysis: Line plot of total CSF SOD1 protein LS geometric mean ratio to baseline values (95% CI) by visit from ANCOVA analysis using MI by baseline plasma NfL level (median) – ITT population



placebo < Median (75.60 pg/mL) n=	20	20	20	19	20	19	20	19	17
tofersen 100 mg < Median (75.60 pg/mL) n=	33	33	32	30	30	31	31	30	23
placebo >= Median (75.60 pg/mL) n=	16	16	15	14	16	14	14	14	11
tofersen 100 mg >= Median (75.60 pg/mL) n=	38	38	38	35	33	34	32	31	31

NOTE 1: Baseline is defined as day 1 value prior to the study drug. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Values below limit of quantitation (BLQ) are set to half of lower limit of quantitation (LLOQ, 15.6 ng/mL) in calculations. Multiple imputation is used for missing data.
NOTE 3: The analysis is based on ANCOVA model with natural log transformed data. The model includes covariates for the corresponding baseline value i.e. log value, baseline disease duration since symptom onset, and use of riluzole or edaravone. The analysis is based on the combined MI datasets from the mITT and non mITT populations.
NOTE 4: The table at the bottom presents the number of subjects with observed non-missing data at each visit.
Abbreviations: CSF = cerebrospinal fluid; SOD1 = superoxide dismutase 1; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square.

Plasma NfL - Change from Study 101 Part C Baseline to Week 28 (Day 197)

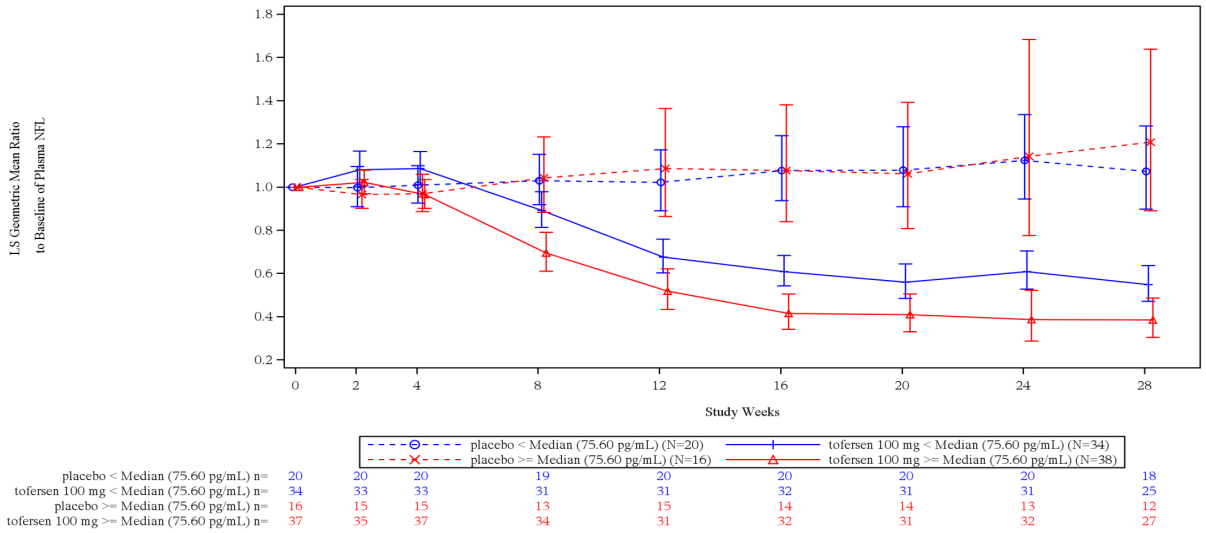
Despite lower levels at Baseline in the non-mITT population, a reduction in plasma NfL levels from baseline to Week 28 were observed in both populations, with a 67% reduction (difference in geometric mean ratios for tofersen to placebo) and nominal $p < 0.0001$ in the mITT population and a 48% reduction (difference in geometric mean ratios for tofersen to placebo) and nominal $p < 0.0001$ in the non-mITT population. A statistically significant decrease of NfL levels has been observed with tofersen in both mITT and non-mITT populations.

In the ITT population, a reduction in plasma NFL levels was also observed at Week 28 in the tofersen group compared to the placebo group (difference in geometric mean ratios for tofersen to placebo of 60% and *post hoc* nominal $p < 0.0001$).

It should be noted however, that neural injury biomarkers of the neurofilament heavy and light chain are the most promising biomarkers in neurodegeneration and have been used in several studies up to now. NfL is released from neurons following axonal injury and can be regarded as a measure of ongoing neurodegeneration. However, NfL and NfH are considered as non-specific, since they are related to the neuronal damage in a variety of neurodegenerative conditions including ALS, Alzheimer's disease,

Multiple Sclerosis and Huntington's disease. Thompson et al published in 2022 their work with Chitinase-1 and NFL and their analysis offers support for the routine inclusion of plasma NfL in future therapeutic trials in ALS. As already mentioned, its prognostic and predictive value is not fully established yet. It should also be noted that there is enough precedence indicating that good results in biomarkers do not always translate into favourable results in clinical endpoints.

Figure 12: Line Plot of Plasma NfL Adjusted Geometric Mean Ratio to Baseline Values (95% CI) by Visit from ANCOVA Analysis Using MI by Baseline Plasma NfL Level (Median) – ITT Population 233AS101 Part C: Secondary endpoint subgroup analysis: Line plot of plasma NfL LS geometric mean ratio to baseline values (95% CI) by visit from ANCOVA analysis using MI by baseline plasma NfL level (median) - ITT population



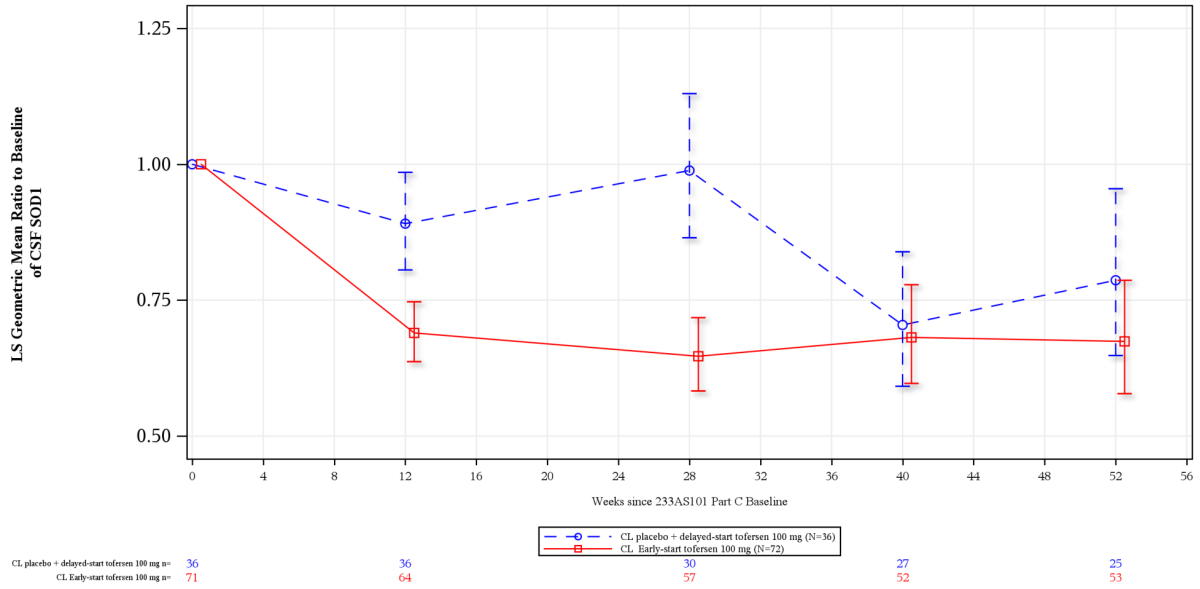
2.6.5.3.2. Biomarkers from Study 101 Part C and Study 102 Week 52 (Day 365) and week 104

Total CSF SOD1 – Study 101 Part C and Study 102 (weeks 52 and 104)

According to the applicant, given the underlying pathophysiology of SOD1-ALS and tofersen's mechanism of action, total CSF SOD1 protein was assessed as an indirect measure of target engagement. In Study 101 Part C, reductions in total CSF SOD1 became apparent by approximately Week 8, consistent with the time required to reach steady state of tofersen and the estimated half-life of SOD1 protein (each approximately 1 month). At Week 28 in the ITT population, a reduction in total CSF SOD1 protein of 35% (geometric mean ratio to baseline) in the tofersen group and a decrease of 2% in the placebo group were observed (difference in geometric mean ratios for tofersen to placebo: 34%; nominal $p < 0.0001$).

In the integrated analysis of Study 101 and Study 102, reductions in total CSF SOD1 in the early-start tofersen group were sustained for 52 weeks of follow-up; similar reductions were observed with later initiation of tofersen in the placebo/delayed-start tofersen group.

Figure 13: 233AS101 and 233AS102: Line Plot of Total CSF SOD1 Adjusted Geometric Mean Ratio to Study 101 Part C Baseline (95% CI) by Visit to Week 52 from ANCOVA Analysis Using MI - ITT Population
233AS101 and 233AS102 ISE: Line plot of total CSF SOD1 protein LS geometric mean ratio to baseline values (95% CI) by time point from ANCOVA analysis using MI - ITT population

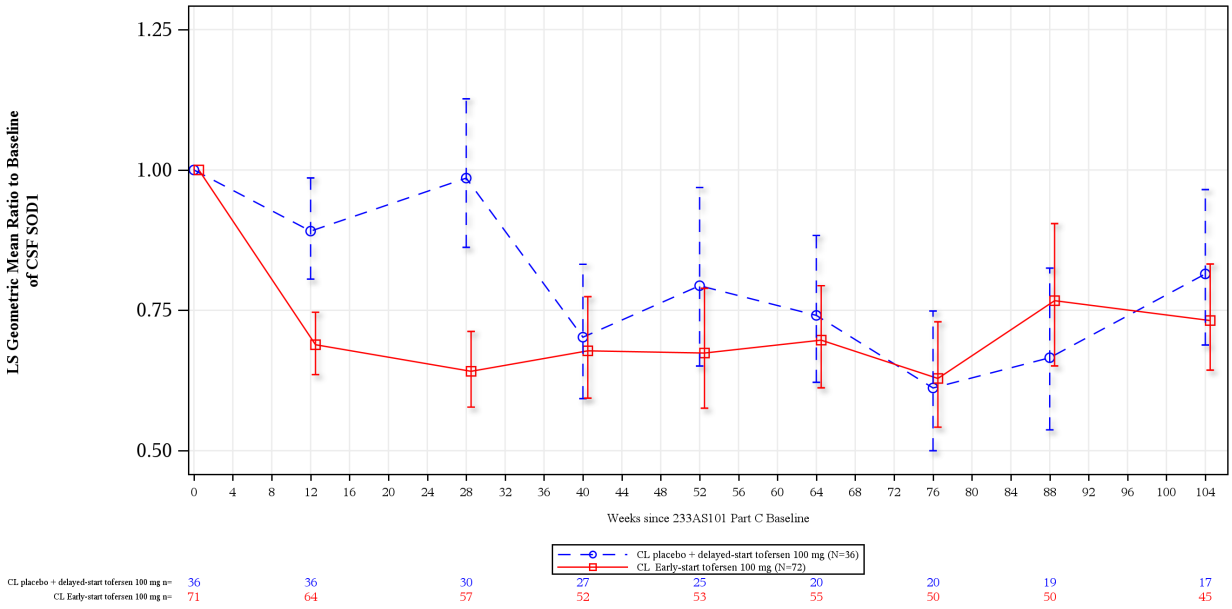


NOTE 1: Baseline is defined as day 1 value prior to the study drug. If day 1 value is missing, the non missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Lower limit of quantitation (LLOQ) is 15.6 ng/mL.
NOTE 3: Multiple imputation including treatment group, use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data.
NOTE 4: The analysis is based on ANCOVA model with natural log transformed data. The model includes covariates for the corresponding baseline value i.e. log value, and use of riluzole or edaravone.
Abbreviations: CSF = cerebrospinal fluid; SOD1 = superoxide dismutase 1; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square.

In the ITT population, the geometric mean ration to baseline was 0.67 for the early start tofersen group (N=72) and 0.79 for the Placebo→ delayed-start tofersen (N=36), indicating reductions of 30% and 20%, respectively. For the pooled ABL population a similar geometric mean ratio to baseline of 0.76 was observed.

Results from the reduction of SOD1 causative protein by tofersen have been collected up to 2 years (week 104). Evaluation of the total CSF SOD1 protein is thought an indirect measure of target engagement. As mentioned above SOD1 is considered as directly related to the pathogenicity of ALS. Reductions of SOD1 protein level up to 50% have been reported in the literature (Berdynski et al 2022). It has been also reported in the literature that gene silencing strategies targeting SOD1 may represent effective approaches for familial and sporadic ALS-related neurodegeneration (Abati et al 2020).

Figure 14: 233AS101 and 233AS102 ISE: Line plot of total CSF SOD1 protein LS geometric mean ratio to baseline values (95% CI) by time point (up to week 104) from ANCOVA analysis using MI - ITT population



NOTE 1: Baseline is defined as day 1 value prior to the study drug. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Lower limit of quantitation (LLOQ) is 15.6 ng/mL. Values below limit of quantitation (BLQ) are set to half of LLOQ in calculations.
NOTE 3: Multiple imputation including treatment group, use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data.
NOTE 4: The analysis is based on ANCOVA model with natural log transformed data. The model includes covariates for the corresponding baseline value i.e. log value, and use of riluzole or edaravone.
Abbreviations: CSF = cerebrospinal fluid; SOD1 = superoxide dismutase 1; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square.

It is observed that the reduction of SOD1 in the case of the early start tofersen treatment group achieves a noticeable decrease at week 12 and reaches the lowest point at week 28, after which it remains at decreased levels until week 104. In the case of the delayed start tofersen treatment group the levels until week 28 remain high and close to the baseline levels. After week 28 (when tofersen treatment started in this group) there is a decrease until week 40 and then SOD1 levels for this delayed start group remain at approximately the same low levels as the early-start group.

2.6.5.3.3. Neurofilament - Study 101 Part C and Study 102 (week 52)

Neurofilaments are intermediate filaments that are uniquely expressed in neurons and are a major constituent of the cytoskeletal scaffold of the CNS and PNS neurons. Neurofilaments are heteropolymers composed of the light, middle, and heavy chains, plus α internexin in the CNS and peripherin in the peripheral nervous system.

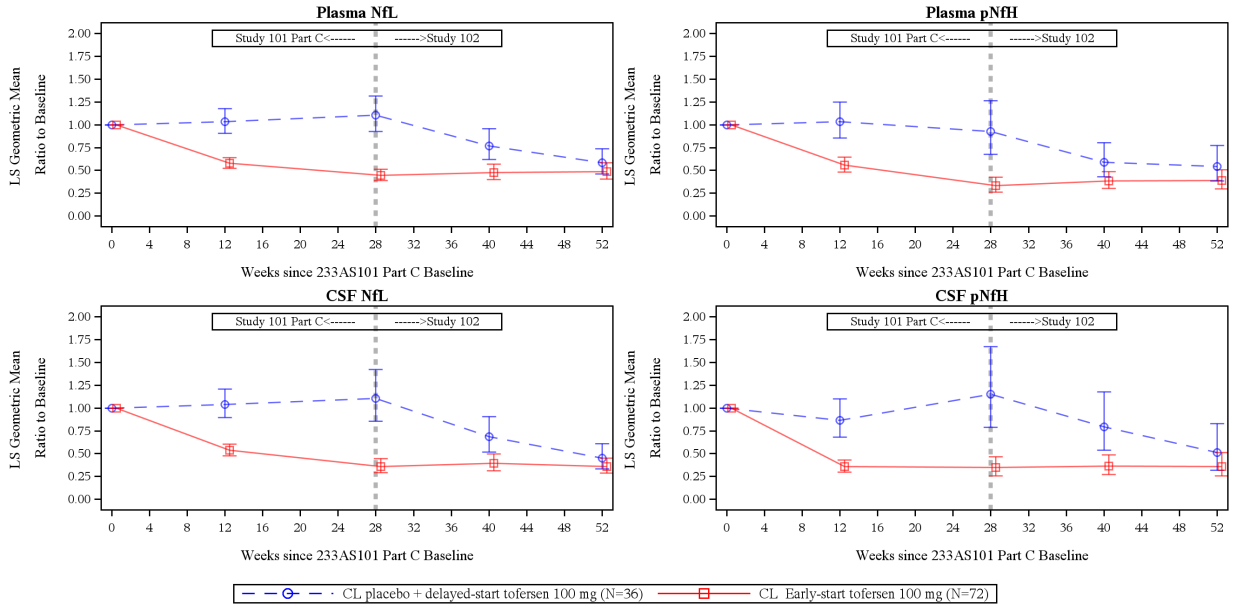
Elevated neurofilament levels have been described in a variety of neurological conditions characterized by neuroaxonal damage, including neurodegenerative diseases and diseases with other pathophysiological processes. Despite the non-specificity, neurofilament levels in ALS are among the highest across neurodegenerative diseases, with levels 7- to 10-fold higher than in neurologically healthy controls.

Longitudinal increases in neurofilament levels were observed before emergence of clinically manifest ALS. Serum NfL levels were elevated 6 to 12 months prior to onset of signs or symptoms of ALS in SOD1 A5V mutation carriers.

According to the applicant, levels of neurofilament have been found to correlate with the rate of disease progression, as measured by the rate of decline on ALSFRS-R from symptom onset to the time of the neurofilament measurement. In other neurological diseases including spinal muscular atrophy and relapsing multiple sclerosis, neurofilament levels have been reduced in response to treatments associated with disease-modifying effects.

In Study 101 Part C and Study 102 (up to week 52), tofersen-driven sustained reductions in neurofilament emerged by week 12 following tofersen administration and preceded the clinical effects seen. At Week 28 of Study 101 Part C, plasma NfL was reduced by 55% (geometric mean ratio to baseline) in the tofersen-treated participants, compared to a 12% increase in placebo-treated participants (difference in geometric mean ratios for tofersen to placebo: 60%; post hoc nominal p < 0.0001) in the ITT population.

Figure 15: 233AS101 Part C and 233AS102 ISE: Line Plot of Plasma NfL, CSF NfL, Plasma pNfH, CSF pNfH LS Geometric Mean Ratio to Baseline Values (95% CI) Over 52 Weeks From 233AS101 Baseline: ANCOVA Analysis Using MI - ITT Population



NOTE 1: Baseline is defined as day 1 value prior to the study drug. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Values below limit of quantitation (BLQ) are set to half of LLOQ in calculations.
NOTE 3: Multiple imputation including treatment group, use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data. An extreme value of 477 pg/mL in plasma NfL is set to missing and is imputed with multiple imputation in the ANCOVA analysis.
NOTE 4: The analysis is based on ANCOVA model with natural log transformed data. The model includes covariates for the corresponding baseline value i.e. log value, and use of riluzole or edaravone.
Abbreviations: CSF = Cerebral spinal fluid; NfL = Neurofilament light chain; pNfH = Phosphorylated neurofilament heavy chain

In the case of early start tofersen treatment group, a significant reduction of NfL has been noted, reaching a maximum at around week 12 and from then onwards remaining at the same low levels. Although NfL is not yet a validated surrogate endpoint, its role is increasingly acknowledged, and the findings are supportive that it is a reliable biomarker in ALS (see Discussion of Clinical Efficacy).

2.6.5.4. Summary of main efficacy results

The following modified tables summarise the efficacy results from the main studies supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 15: Summary of efficacy for Study 233AS101 Part C (VALOR) (week 28)

<table>
<tr><td colspan="5">Title: A Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis and Confirmed Superoxide Dismutase 1 Mutation</td></tr>
<tr><td>Study identifier</td><td colspan="4">Protocol number: 233AS101 Part C
EudraCT number: 2015-004098-33
ClinicalTrials.gov identifier (NCT number): NCT02623699</td></tr>
<tr><td rowspan="2">Design</td><td colspan="4">A multicenter, randomized, double-blind, placebo-controlled Phase 3 study to examine the efficacy, safety, tolerability, PK, and PD of tofersen administered by intrathecal bolus injection to adult participants with ALS associated with mutations in the SOD1 gene.
Participants were randomized to receive tofersen 100 mg or placebo in a 2:1 (active:placebo) ratio for 28 weeks (197 days) at 32 sites globally. Randomisation was stratified by disease progression subgroups (‘enriched’ ‘other’) and riluzole/edaravone use.
Participants who completed the study had the opportunity to be screened for an open-label long-term extension study, Study 233AS102.</td></tr>
<tr><td>Duration of main phase:

Duration of Run-in phase:

Duration of Extension phase:</td><td colspan="3">32 to 36 weeks
4-weeks screening period
24-week treatment period
4-to 8-week follow-up period as follows:
- Participants who enrolled (uninterrupted) in the LTE study (233AS102), the Week 28 Visit served as the EOS visit.
- Participants with delayed enrolment in the LTE study, the Week 32 Visit served as the EOS Visit (either in person or by telephone contact).
- Participants who did not enroll in the LTE study, the Week 32 Visit served as the EOS Visit (either in person or by telephone contact).
Not applicable
No extension in the Study 101 protocol; Study 102 is the extension study for participants who completed Study 101 and is ~3.5-7 years in duration depending on timing of enrollment.</td></tr>
<tr><td>Hypothesis</td><td colspan="4">Superiority</td></tr>
<tr><td rowspan="2">Treatments groups</td><td>tofersen</td><td colspan="3">Treatment: 100 mg tofersen administered via intrathecal bolus injection over 1 to 3 minutes
Duration: 3 loading doses once every 2 weeks (Day 1, 15, 29), followed by 5 maintenance doses once every 28 days (4 weeks).
Number randomised: 72</td></tr>
<tr><td>placebo</td><td colspan="3">Treatment: Placebo consisted of artificial CSF administered by intrathecal bolus over 1 to 3 minutes, following the same dosing regimen as tofersen.
Duration: 3 loading doses once every 2 weeks (Day 1, 15, 29), followed by 5 maintenance doses once every 28 days (4 weeks).
Number randomised: 36</td></tr>
<tr><td>Endpoints and definitions</td><td colspan="2">Primary:

Change from baseline to Week 28 in ALSFRS-R</td><td>ALSFRS-R</td><td>Change from baseline in ALSFRS-R total score in the mITT population</td></tr>
</table>

	Secondary: Change from baseline to Week 28 in total SOD1 protein concentration in CSF	Total CSF SOD1 protein	Change from baseline in total SOD1 protein concentration in CSF in the mITT population. Expressed as a ratio (Week 28 to baseline) as it is analysed on logarithmic scale. Also formally tested in non mITT population.
	Secondary: Change from baseline to Week 28 in plasma NfL	Plasma NfL	Change from baseline in NfL concentration in plasma in the mITT population. Expressed as a ratio (Week 28 to baseline as it is analysed on logarithmic scale
	Secondary: Change from baseline to Week 28 in percent predicted SVC	SVC	Measure of respiratory function. Change from baseline in percent predicted SVC in the mITT population, using maximum i.e. best effort in predicted SVC at the visit as assessed using a spirometer, which is adjusted by sex, age, and height. Percent predicted SVC is calculated as observed SVC divided by predicted SVC
	Secondary: Change from baseline to Week 28 in HHD megascore	HHD	Measure of muscle strength. Change from baseline in HHD megascore in the mITT population, which is the average of the normalized Z scores for muscle strength in 16 muscle groups in upper and lower extremities on both right and left sides of the body.
	Secondary: Time to death or PV	Time to death or PV	Time to earliest of death or PV from first dose in Study 101 Part C in the mITT population based on independent adjudication, where PV is defined as ≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥21 consecutive days. Participants who do not meet the endpoint definition are censored at the last contact date in Study 101 Part C
Database lock	16th August 2021		

Results and Analysis

Analysis description	**Primary Endpoint Analysis**				
Analysis population and time point description		*mITT population*		*ITT population* *(Post hoc analyses adjusting for baseline plasma NfL as a covariate)*	
	Treatment group	placebo	Tofersen	Placebo	tofersen
	N number	21	39	36	72
Endpoint: **Change from baseline to Week 28 in ALSFRS-R**					

Descriptive statistics and estimate variability	Adjusted mean	-8.14	-6.98	-6.2	-4.1
Effect estimate per comparison tofersen vs placebo	Adjusted mean difference 95% CI p-value (Joint rank+MI) Nominal p–value (ANCOVA + MI)	1.2 -3.2, 5.5 0.9689 0.5998		2.1 -0.3, 4.5 0.5015 0.0904	
Analysis description	**Secondary endpoint analysis**				
Analysis population and time point description		*mITT*		*ITT population* *(Post hoc analyses adjusting for baseline plasma NfL as a covariate)*	
	Treatment group	Placebo	tofersen	Placebo	tofersen
	N Number	21	39	36	72
Endpoint: **Change from baseline to Week 28 in total CSF SOD1 protein**					
Descriptive statistics and estimate variability	Adjusted GMR to baseline	1.16	0.71	0.98	0.65
Effect estimate per comparison tofersen vs placebo	Difference in GMR (tofersen: placebo) 95% CI Nominal p-value (ANCOVA +MI)	0.62 0.49, 0.78 <0.0001		0.66 0.57, 0.77 <0.0001	
Endpoint: **Change from baseline to Week 28 in Plasma NfL**					
Descriptive statistics and estimate variability	Adjusted GMR to baseline	1.20	0.40	1.12	0.45
Effect estimate per comparison tofersen vs placebo	Difference in GMR (tofersen: placebo) 95% CI Nominal p-value (ANCOVA +MI)	0.33 0.25, 0.45 <0.0001		0.40 0.33, 0.49 <0.0001	
Endpoint: **Change from baseline to Week 28 in SVC**					

Descriptive statistics and estimate variability	Adjusted mean	-22.20	-14.31	-15.82	-7.34
Effect estimate per comparison tofersen vs placebo	Adjusted mean difference 95% CI Nominal p-value (Joint rank+MI) Nominal p–value (ANCOVA+MI)	7.9 -3.5, 19.3 0.3233 0.1755		8.5 1.8, 15.2 0.0689 0.0128	
Endpoint: **Change from baseline to Week 28 in HHD Megascore**					
Descriptive statistics and estimate variability	Adjusted mean	-0.37	-0.34	-0.32	-0.23
Effect estimate per comparison tofersen vs placebo	Adjusted mean difference 95% CI Nominal p–value (ANCOVA+MI)	0.02 -0.21, 0.26 0.8390		0.10 -0.04, 0.23 0.1547	
Endpoint: **Time to death or PV**					
Descriptive statistics and estimate variability	Number of events/Total number subjects (%) Median	2/21 (9.5%) NE	4/39 (10.3%) NE	2/36 (5.6%) NE	4/72 (5.6%) NE
Effect estimate per comparison tofersen vs placebo	Not available as median time is not estimable due to limited number of events				
Endpoint: **Time to death**					
Descriptive statistics and estimate variability	Number of events/Total number subjects (%) Median	N NE	N NE	N NE	N NE
Effect estimate per comparison tofersen vs placebo	Not available as median time is not estimable due to limited number of events				
Analysis description	**Exploratory analysis**				

Analysis population and time point description	Analyses for exploratory endpoints including quality of life measures were prespecified in the mITT population for changes from baseline to Week 28. Following the readout, post hoc analyses were conducted for quality-of-life measures in disease progression subgroups based on baseline plasma NfL as well as analyses adjusting for baseline NfL as a covariate in the ITT population.				
		mITT		*ITT population* *(Post hoc analyses adjusting for baseline plasma NfL as a covariate)*	
	Treatment group	placebo	tofersen	Placebo	tofersen
	N number	21	36	36	72
Endpoint: **Change from baseline to Week 28 in ALSAQ-5 total score**					
Descriptive statistics and estimate variability	Adjusted mean	15.6	10.0	12.6	6.9
Effect estimate per comparison tofersen vs placebo	Adjusted mean difference 95% CI Nominal p–value (ANCOVA+MI)	-5.6 (-15.6, 4.4) 0.2715		-5.7 (-11.8, 0.4) 0.0668	
Endpoint: **Change from baseline to Week 28 in FSS**					
Descriptive statistics and estimate variability	Adjusted mean	10.46	5.56	6.3	3.9
Effect estimate per comparison tofersen vs placebo	Adjusted mean difference 95% CI Nominal p–value (ANCOVA+MI)	-4.91 (-11.23, 1.42) 0.1283		-2.4 (-7.45, 2.55) 0.3365	
Endpoint: **Change from baseline to Week 28 in EQ-5D-5L Utility Score**					
Descriptive statistics and estimate variability	Adjusted mean	-0.35	-0.16	-0.21	0.08
Effect estimate per comparison tofersen vs placebo	Adjusted mean difference 95% CI Nominal p–value (ANCOVA+MI)	0.20 (0.06, 0.33) 0.0043		0.14 (0.05, 0.23) 0.0029	

N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

Table 16: Summary of integrated efficacy in Study 101 Part C and Study 102 (on-going)

Title: Study 101 Part C: A Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis and Confirmed Superoxide Dismutase 1 Mutation Study 102: An Extension Study to Assess the Long-Term Safety, Tolerability, Pharmacokinetics, and Effect on Disease Progression of BIIB067 Administered to Previously Treated Adults with Amyotrophic Lateral Sclerosis Caused by Superoxide Dismutase 1 Mutation		
Study identifier	Protocol number: 233AS101 Part C EudraCT number: 2015-004098-33 ClinicalTrials.gov identifier (NCT number): NCT02623699	
Design	Study 101 Part C is a multicenter, randomized, double-blind, placebo-controlled Phase 3 study to examine the efficacy, safety, tolerability, PK, and PD of tofersen administered by intrathecal bolus injection to adult participants with ALS associated with mutations in the SOD1 gene. Participants were randomized to receive tofersen 100 mg or placebo in a 2:1 (active:placebo) ratio for 28 weeks (197 days) at 32 sites globally. Participants who completed the study had the opportunity to be screened for an open-label long-term extension study, Study 233AS102. Study 102 is an ongoing, multicenter, open-label, long-term extension study to assess the long-term safety, tolerability, PK, PD, and efficacy of tofersen administered by IT injection to participants with SOD1-ALS who completed Part C of Study 233AS101. Efficacy data from Study 233AS101 Part C and an interim data cut of Study 102 performed on 16 January 2022 are described herein. Crossover, or evaluation of "early-start" tofersen vs "delayed-start" tofersen was prospectively incorporated into the design of the tofersen CDP. treatment sequences for both arms were prespecified. The analysis of early-start tofersen versus delayed-start tofersen allows the ITT principle and allows an objective evaluation of the 2 treatment sequences. To preserve the integrity of ongoing data generation, site staff, study participants, and vendors remain blinded to individual treatment assignments from Study 101 Part C.	
	Duration of Extension phase: Follow up period:	Up to 360 weeks 4 weeks
Hypothesis	Superiority	
Treatments groups	Early Start tofersen	Participants who started tofersen 100 mg in Study 101 Part C and had the opportunity to continue on tofersen 100 mg in Study 102
	Placebo/Delayed Start tofersen	Participants who received placebo in Study 101 Part C and had the opportunity to start tofersen 100 mg in
	Participant accountability	A total of 97 participants who completed Study 233AS101 Part C were eligible to enroll in Study 233AS102. Of these 95 participants enrolled in Study 102. The integrated efficacy analyses account for all 108 participants who were randomized in Study 101 Part C.

The same important endpoints as in the case of Study 101 Part C were used. **Please see below.**			
Database lock	Data cut for Study 102 taken 16 January 2022 database lock on 28 February 2022		
Results and Analysis			
Analysis description	**Primary Analysis**		
	ITT population *(adjusting for baseline plasma NfL as a covariate)*		
	Treatment group	Placebo/Delayed -start tofersen	Early-start tofersen
	N number	72	36
Endpoint: **Change from baseline to Week 52 in ALSFRS-R**			
Descriptive statistics and estimate variability	Adjusted mean	-9.5	-6.0
Effect estimate per comparison Early-start vs. placebo/Delayed-start	Adjusted mean difference 95%CI p-value (ANCOVA+MI)	3.5 0.4, 6.7 0.0272	
Endpoint: **Change from baseline to Week 52 in total SOD1 protein concentration in CSF**			
Descriptive statistics and estimate variability	Adjusted GMR to baseline	0.79	0.67
Endpoint: **Change from baseline to Week 52 in plasma NfL**			
Descriptive statistics and estimate variability	Adjusted GMR to baseline	0.59	0.49
Endpoint: **Change from baseline to Week 52 in percent predicted SVC**			
Descriptive statistics and estimate variability	Adjusted mean	-18.6	-9.4
Effect estimate per comparison Early-start vs. placebo/Delayed-start	Adjusted mean difference 95%CI p-value (ANCOVA+MI)	9.2 1.7, 16.6 0.0159	
Endpoint: **Change from baseline to Week 52 in HHD megascore**			
Descriptive statistics and estimate variability	Adjusted mean	-0.45	-0.17
Effect estimate per comparison Early-start vs. placebo/Delayed-start	Adjusted mean difference 95%CI p-value (ANCOVA+MI)	0.28 0.047, 0.517 0.0186	

Endpoint: **Time to death or PV**			
Descriptive statistics and estimate variability	Number of events/Total number subjects (%) Median	8/36 (22.2%) NE	12/72 (16.7%) NE
Effect estimate per comparison Early-start vs. placebo/Delayed-start	Hazard ratio 95%CI Cox regression p-value Log-rank test p-value	0.36 0.137, 0.941 0.0373 0.0687	
Endpoint: **Time to death**			
Descriptive statistics and estimate variability	Number of events/Total number subjects (%) Median	6/36 (16.7%) NE	8/72 (11.1%) NE
Effect estimate per comparison Early-start vs. placebo/Delayed-start	Hazard ratio 95%CI Cox regression p-value Log-rank test p-value	0.27 0.084, 0.890 0.0313 0.0879	

*This Table was provided by the applicant and should be read in conjunction with the additional analyses and the discussion of the results in section 3.3.4.6. Analysis performed across trials (pooled analyses and meta-analysis).

2.6.5.5. Clinical studies in special populations

The following information (Table 17) has been retrieved from the Clinical Study 233AS101 Report and the Clinical Study 233AS102 Report Interim 2

Table 17: Clinical studies in special populations

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	14 (9 tofersen, 5 placebo)		
Non Controlled trials	6	2	

However, a subgroup analysis based on age groups, especially those above 65 years of age would not be considered useful in ALS and with the available data on tofersen.

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

Data from the randomized controlled period of Study 101 were integrated with the Study 102 LTE to inform the longer-term benefit/risk of tofersen. The primary focus of the integration was the longitudinal experience of the Study 101 Part C participants across Studies 101 Part C and 102 (pooled group CL) who were grouped as follows:

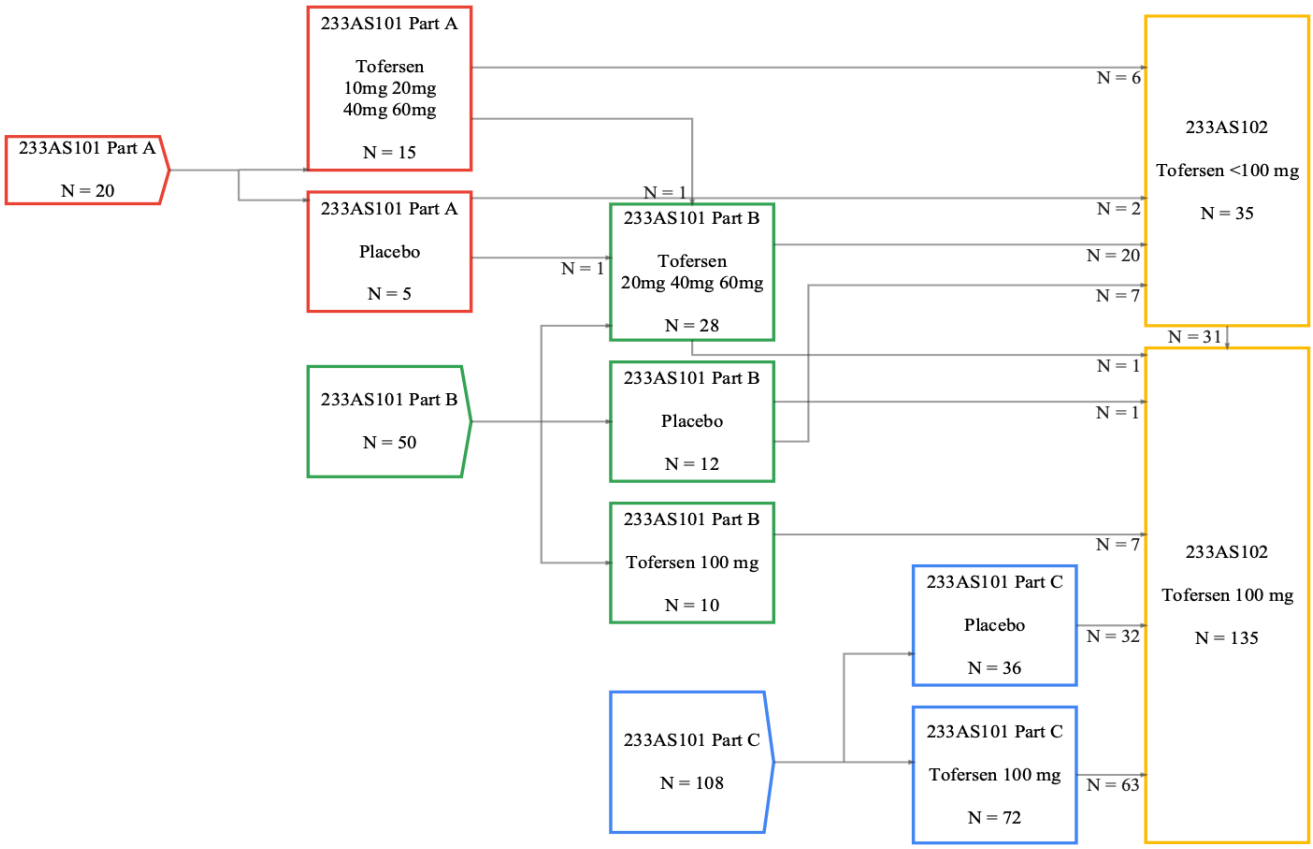
- Early-start participants who started tofersen 100 mg in Study 101 Part C and had the opportunity to continue on tofersen 100 mg in Study 102 (CL1).
- Placebo/delayed-start participants who received placebo in Study 101 Part C and had the opportunity to start tofersen 100 mg in Study 102 (CL2).

Participants

Ninety-five of 108 participants (87%) randomized in Study 101 Part C went on to receive tofersen in Study 102. At the time of the 16 January 2022 interim data cut, 71% (67/95) of these participants remained ongoing in Study 102 (Figure below). An accounting of participant disposition is below in Table below.

The following graph (Figure 16) is showing the participants flow of study 101 (Parts A, B and C) and 102.

Figure 16: Flow of participants through Study 233AS101 and Study 233AS102



Participants enrolled:	N=20 in Part A	N=50 in Part B (including 2 from Part A)	N=108 in Part C
Participants eligible for Study 233AS102:	N=19 from Part A	N=45 from Part B	N=97 from Part C
Participants enrolled in Study 233AS102:	N=8 from Part A	N=36 from Part B	N=95 from Part C

Seventy patients in total were receiving tofersen 100mg before entering the study 102 LTE. From these, 21 patients from study 101 Part C belonged to the mITT and were characterised as Faster Progressing and 28 patients were in the non-mITT population and were characterised as slower progressing. Seven

patients in study 101 Part B and in study 102 received tofersen 100mg. From the parts A and B, 35 patients enrolled in study 102 at tofersen less than 100mg and 9 patients from Part B enrolled in the study 102 at tofersen 100mg (in total 44 patients).

2.6.5.6.1. Pooling of Efficacy Data - 2nd Interim report (data cutoff 16 January 2022) with results up to week 52

The integrated summary has combined efficacy data across clinical studies of tofersen. Two pooled groups of study subjects considered in SAP Integrated Summary of Efficacy, Version No.: 3.0) are summarized in the following Table 18.

Table 18: Definitions of the pooled groups in this integrated summary of efficacy

Pooled Group[1]	Studies Included	Period	Description
CL	233AS101 Part C and 233AS102	Randomized placebo-controlled and long-term	Data of *all* Part C subjects in 233AS101 *and* the data in 233AS102 of 233AS101 Part C subjects who rolled over into the LTE
ABL	233AS101 Part A, B and 233AS102	Randomized placebo-controlled and long-term	Data of *all* 233AS101 Part A/B subjects in 233AS101 *and* the data in 233AS102 of 233AS101 Part A/B subjects who rolled over into the LTE

[1]CL: 233AS101 Part C and LTE; ABL: 233AS101 Part A, B and LTE.

The primary time points of interest for the integration of mITT population are 12 weeks (Day 85), 28 weeks (Day 197), 40 weeks (Day 281), and 52 weeks (Day 365) from 233AS101 Part C baseline. These will also be the primary time points of interest when presenting the integration of overall ITT population of pooled group CL. For by-visit summaries of observed data, all visits with data available up to 52 weeks (Day 365) from 233AS101 Part C baseline will be used.

The primary time points of interest for the integration of non-mITT population are 12 weeks (Day 85), 28 weeks (Day 197), 40 weeks (Day 281), 52 weeks (Day 365), 64 weeks (Day 449), 76 weeks (Day 533), 88 weeks (Day 617), and 104 weeks (Day 729) from 233AS101 Part C baseline. For by-visit summaries of observed data, all visits with data available up to 104 weeks (Day 729) from 233AS101 Part C baseline will be used.

No formal testing was performed. The reported p-values are for exploratory purpose only.

MI was used to impute data up to and including Week 52 for continuous endpoints; ANCOVA+MI analyses were conducted. The model for MI included all participants in the ITT population including treatment, baseline plasma NfL in the original scale, riluzole or edaravone use, the corresponding baseline value and post-baseline values. The ANCOVA analysis used these MI datasets and included treatment as a fixed effect, and adjusted for covariates: baseline plasma NfL, riluzole or edaravone use and the corresponding baseline value. ANCOVA analyses were performed at approximately 3-month time-points. Nominal p-values were presented for the treatment difference at Week 52. Log-transformed data were used for PD and biomarker endpoints. Nominal p-values were presented for the treatment difference at Week 52. In the subgroup analyses, data are presented to Week 104 for the non-mITT population.

Time to death or permanent ventilation and time to death analyses were performed using a Kaplan-Meier. Treatment comparison was based on a log rank test stratified by treatment and median baseline

plasma NfL. A Cox regression model adjusted for baseline plasma NfL, and riluzole or edaravone use was used to obtain the hazard ratio and 95% confidence intervals for each of these endpoints.

Table 19: Participant disposition of integrated analysis of studies 101 Part C and 102 for the ITT population

	ISE Based on Jul 2021 Data Cut		**ISE Based on Jan 2022 Data Cut (week 52)**	
Participants	**Early-start Tofersen 100 mg (n = 72)**	**Placebo/delayed - start Tofersen 100 mg (n = 36)**	**Early-start Tofersen 100 mg (n = 72)**	**Placebo/delayed-start Tofersen 100 mg (n = 36)**
Dosed in Study 101 Part C n (%)	72 (100)	36 (100)	72 (100)	36 (100)
Dosed in Study 102 n (%)	63 (88)	32 (89)	63 (88)	32 (89)
Completed Study 101 Part C but not enrolled in Study 102 n (%)	1 (1)	1 (3)	1 (1)	1 (3)
Ongoing in Study 102 n (%)	54 (75)	22 (61)	49 (68)	18 (50)
Died while on trial n (%)	7 (10)	4 (11)	8 (11)	6 (17)
Withdrew from trial n (%)	17 (24)	13 (36)	22 (31)	17 (47)
- AE	2 (3)	0	2 (3)	0
- Consent withdrawn	3 (4)	3 (8)	4 (6)	4 (11)
- Death	7 (10)	4 (11)	8 (11)	6 (17)
- Disease progression	5 (7)	6 (17)	8 (11)	7 (19)
Post-withdrawal vital status not collected	N/A	N/A	5 (7)	3 (8)
Total deaths including post-withdrawal death[a]	N/A	N/A	12 (17)	11 (31)

[a] Post withdrawal vital status was collected retrospectively for participants who withdrew from Study 101 Part C or Study 102 or who completed Study 101 Part C and did not enrol in Study 102.

The population which is more in the focus in study 102 LTE are patients who participated in study 101 Part C i.e. CL population, since it is the population for which double blind data are available and there was not a wash out period as in the case of ABL patients.

ALSFRS-R Total Score

According to the applicant, analyses for Study 101 Part C performed in the overall ITT population, adjusting for baseline plasma NfL as a covariate rather than disease duration in both the analysis model and imputation model, show a greater treatment difference in favor of tofersen compared to the prespecified primary analysis.

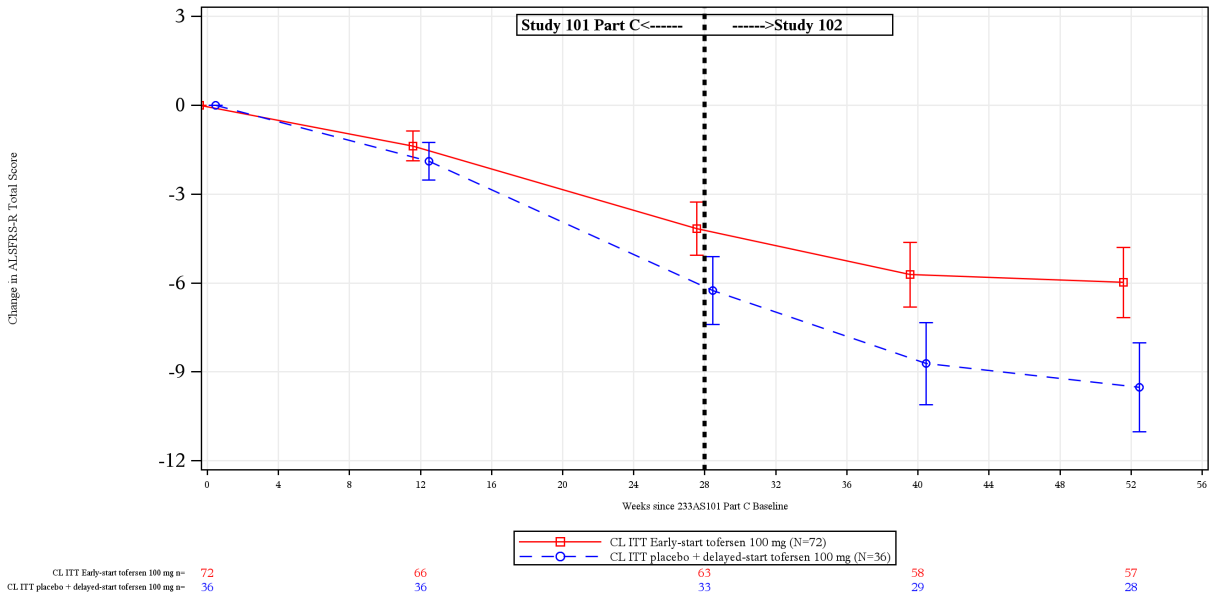
Table 20: Change in ALSFRS-R total score from study 101 Part C baseline up to week 52 - ITT population

Endpoint **(Post hoc analyses with baseline plasma NfL as a covariate)**	**Study 101 Part C** Tofersen (n=72) vs. placebo (n=36) -Change from baseline to Week 28	**Studies 101 Part C and 102 (ISE SAP V3.0)** Early-start tofersen (n=72) vs. placebo/delayed-start tofersen (n=36)- Change from baseline to Week 52
Change from Baseline on ALSFRS-R Total Score		
Adjusted means: Tof, Placebo	-4.1, -6.2	-6.0, -9.5
Tofersen-placebo: adjusted mean difference (95% CI)	**2.1 (-0.33, 4.54)**	**3.5 (0.4, 6.7)**
p-value (ANCOVA+MI)	**0.0904**	**0.0272**

NOTE 1: Lower scores depict a worsening in function. Post hoc analyses are presented for Study 101 Part C; analysis for Studies 101 Part C and 102 is based on January 2022 data cut and was prespecified in ISE SAP Version 3.0. All p-values are nominal.
NOTE 2: The multiple imputation model is based on all participants in the ITT population and includes baseline plasma NfL, treatment, use of riluzole or edaravone, relevant baseline score, and post-baseline values.
NOTE 3: Adjusted means, treatment difference and corresponding 95% CIs and nominal p-values are obtained from the ANCOVA model for change from baseline in conjunction with MI. Joint rank p-values are obtained from the ANCOVA model for ranked scores

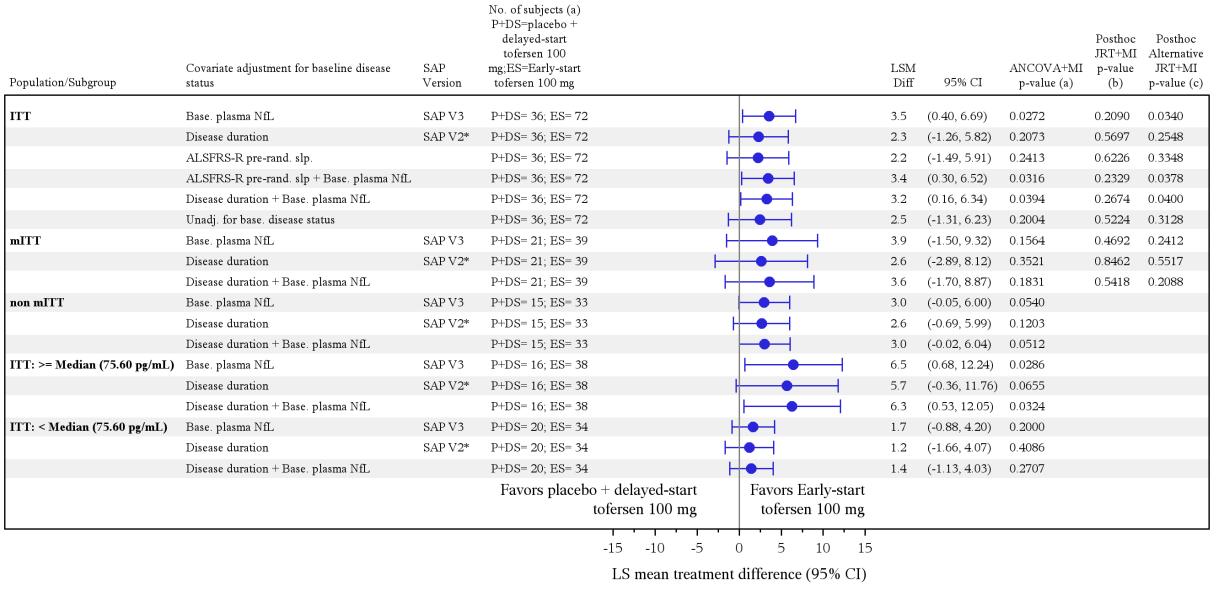
where deaths are ranked the lowest in conjunction with MI for handling missing data due to withdrawals other than death. The original joint rank method is based on the ANCOVA for ranked score for the change from baseline and includes original scale values for continuous covariates; the alternative joint rank method is based on the ANCOVA for change of ranked score pre- and post-treatment and includes ranked covariates for continuous variables. The ANCOVA models include treatment as a fixed effect and adjust for the following covariates: baseline plasma NfL, relevant baseline score, and use of riluzole or edaravone.

Figure 17: 233AS101 and 233AS102 ISE: Line Plot of ALSFRS-R total score adjusted mean change from baseline values ± SE by timepoint from ANCOVA analysis using MI for pooled group CL - ITT population (week 52)



NOTE 1: Baseline is defined as day 1 value prior to the study drug and presented as Day 1. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Multiple imputation including treatment group, use of riluzole or edaravone, baseline plasma NfL, and the relevant baseline and postbaseline values for the endpoint is used for missing data.
NOTE 3: For non-Japanese subjects, the Global ALSFRS-R is used. For Japanese subjects, the Japanese (Ohashi) ALSFRS-R is used except for Q5a and Q11 where the Japanese translated Global ALSFRS-R is used. A positive change indicates an improvement.
NOTE 4: LS means are obtained from the ANCOVA model with treatment included as a fixed effect and adjusted for the following covariates: baseline plasma NfL, baseline ALSFRS-R total score, and use of riluzole or edaravone.
Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised; NfL = neurofilament light chain; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square.

Figure 18: 233AS101 and 233AS102 ISE: Forest plot of ALSFRS-R total score change from baseline to week 52



NOTE 1: SAP V2.0 is the integrated efficacy SAP that was finalized prior to the final database lock for 233AS101 and interim lock for 233AS102 based on 16 July 2021 data cut; prespecified analyses were primarily based on disease progression subgroups and adjusted for disease duration since symptom onset. SAP V3.0 was an amendment to the integrated efficacy SAP following the initial data readout

and incorporated plasma NfL as a covariate with the focus on the ITT population. SAP V3.0 was finalized prior the interim lock for 233AS102 based on 16 January 2022 data cut.
NOTE 2: For ITT population, multiple imputation including the specified covariate(s), use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include the specified covariate(s), baseline ALSFRS-R, and use of riluzole or edaravone.
NOTE 3: For the analyses including disease duration since symptom onset in mITT and non-mITT population, multiple imputation including use of riluzole or edaravone and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include covariates for baseline disease duration since symptom onset, baseline ALSFRS-R and use of riluzole or edaravone. For the other analyses in mITT and non-mITT population, multiple imputation including baseline plasma NfL, use of riluzole or edaravone and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include covariates for the specified covariate(s), baseline plasma NfL, baseline ALSFRS-R and use of riluzole or edaravone.
* No statistical testing was prespecified in SAP Version 2; all p-values are post hoc for SAP V2 analyses.
(a) From the listed ANCOVA analysis based on change from baseline.
(b) From the ANCOVA on ranked scores; deaths are ranked the lowest; MI is used for handling missing data due to withdrawals other than death.
(c) From the ANCOVA on change of ranked scores pre- and post-treatment; deaths are ranked the lowest; MI is used for handling missing data due to withdrawals other than death.
Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square; P+DS = placebo + delayed-start tofersen 100 mg; ES = Early-start tofersen 100 mg.

The analysis (data cutoff 16 January 2022) of the pooled double-blind data from study Part C and the open-label data from study 102 LTE up to week 52, have shown a difference between the early and the placebo/delayed start tofersen treatment group. However, these results from exploratory analyses cannot be considered a robust demonstration of efficacy. A clear and statistically significant difference may have been shown if the duration of the study with a placebo control was 52 weeks. Nevertheless, the Forest plot with all the analyses of ALSFRS-R is showing in all subgroups a trend in favour of tofersen.

The comparison of delayed start of tofersen with early start of tofersen is not an ideal basis to support deciding on B/R of tofersen but it still provides the only randomised comparison of longer-term tofersen treatment vs a control. As for the 28-week analysis, from a regulatory point of view, the treatment effect irrespectively of treatment discontinuation and considering the value 0 as the true value after death is of primary relevance. However, although reference-based imputation analyses were also conducted for the 52-week analysis time point, the interpretation of these analyses is not straightforward, as the reference group is also actively treated in contrast to patients who discontinued treatment. Nevertheless, the analysis is at least more conservative than the analysis based on a missing at random assumption when targeting the treatment effect of primary regulatory relevance.

SVC

Analyses for Study 101 Part C performed in the overall ITT population, adjusting for baseline NfL as a covariate rather than disease duration and baseline ALSFRS-R score, show a nominally statistically significant 8.5 percent-predicted treatment difference at Week 28 in favor of tofersen (approximately 54% slowing of decline for tofersen relative to placebo).

Over the longer term in the ITT population, participants in the placebo/delayed-start arm experienced a nominally statistically significant greater decline from baseline to Week 52 on percent-predicted SVC than the early-start participants (9.2 percent-predicted treatment difference; 95% CI: 1.7, 16.6;). Notably in the placebo/delayed-start group, declines appeared to stabilize after Week 40, approximately 12 weeks after initiation of tofersen and the time point at which maximum biological activity would be expected.

According to the applicant, the 8.5 and 9.2 percent-predicted differences observed between treatment groups at 28 and 52 weeks, respectively, are highly clinically relevant.

As in the case of ALSFRS-R the combined double blind and open label data have shown a difference between the early and the placebo/delayed start tofersen treatment group in the percent predicted SVC, albeit small.

HDD Megascore

Change in HHD megascore over 28 weeks was assessed in the mITT population as a key secondary endpoint in Study 101 Part C. In this subgroup, a modest difference of 0.02 favoring tofersen was observed (95% CI: -0.21 to 0.26); ANCOVA+MI nominal p = 0.8390.

Though not clearly discernible until Week 52, the stabilisation and, in some cases, improvement in muscle strength associated with tofersen treatment are completely inconsistent with the natural history of ALS.

Similarly, to SVC the combined double blind and open label data have shown a very small difference between the early and the placebo/delayed start tofersen treatment group in HDD.

Survival (data cutoff 16 January 2022; week 52)

Survival and other time to event endpoints with data cut-off 16 January 2022 were provided but are not described and discussed here because more mature data with data cut-off 28 February 2023 at week 104 are available (see below).

- *Raw Data Pilot Project Analysis 1st Evaluation phase (data cutoff 16 January 2022; week 52)*

The following contains Table results of the survival analyses outlined in the SAP. Survival analyses are performed with the endpoint used by the applicant, death, or PV assistance. The analyses are similar to the analyses performed by the applicant but with four model adjustments, as represented by the last four rows in the table below. The first row is similar to the analysis by the applicant and is included as a reference.

In the second row, the survival analysis is restricted to an observation period of 52 weeks from baseline and adjusted for baseline NfL concentration, riluzole or edaravone use, and PV assistance defined as 21 consecutive days of ventilation. In the third and fourth rows, results of sensitivity analyses are shown with PV assistance definitions of "7 consecutive days of ventilation" and "10 consecutive days of ventilation" (always keeping steady the covariate of ≥ 22 hours of mechanical ventilation invasive or noninvasive), respectively. These analyses are adjusted for baseline NfL concentration and riluzole and edaravone use. Lastly, in the fifth row, results are shown without adjustment for NfL concentration but with adjustment for riluzole and edaravone use and a PV assistance definition of 21 consecutive days of ventilation.

Table 21: (the Raw Data Pilot Project): Results from survival analyses of time to death or permanent ventilation PV up to week 52 (16 January 2022 data cutoff).

Description	Hazard ratio (95% CI)	Wald test	Log-rank test	Events of total in early tofersen group	Percentage, early tofersen group	Events of total in delayed tofersen group	Percentage, delayed tofersen group
Unrestricted analysis (as the Applicant)	0.36 (0.137, 0.941)	0.0373	0.0687	12/72	16.7%	8/36	22.2%
Restriction to 52 weeks	0.87 (0.213, 3.582)	0.8508	0.8856	7/72	9.7%	3/36	8.3%
7 days	0.38 (0.151, 0.938)	0.0359	0.0440	12/72	16.7%	9/36	25.0%
10 days	0.36 (0.142, 0.892)	0.0275	0.0402	12/72	16.7%	9/36	25.0%
No adjustment for NfL	0.61 (0.248, 1.506)	0.2849	0.2773	12/72	16.7%	8/36	22.2%

HR=hazard ratio, CI=confidence interval.

The limit of 21 consecutive days of ≥ 22 hours of permanent mechanical ventilation was considered conservative, taking into consideration publicly available information on clinical trials conducted in the field of ALS. Additional analyses with 7 and 10 consecutive days of ≥ 22 hours of permanent ventilation were performed with the Raw Data Pilot Project. However, due to the limited number of events there was no difference in the results whether 7, 10 or 21 consecutive days of ventilation were chosen. The percentage of events (time to death or permanent ventilation) in the early tofersen treatment group was 16.7% compared to the percentage of 22.2% events in the placebo/delayed tofersen treatment. Hazard ratio (HR) was in the region of 0.36-0.38.

These results could have been more convincing, provided that they had derived entirely from controlled data and that the functional endpoints would be significantly in favour of tofersen.

The results from a Cox model adjusting for baseline NfL and riluzole/edaravone use, and a log-rank test stratifying for median baseline NfL are presented. The adjustment for baseline NfL (continuous scale) was not included in V2 of the SAP but only in V3 when results of part C were already known. The number of events was overall small such that single events may have a relevant influence on the results, raising concerns on the robustness of the analysis.

For the analysis of time to death or permanent ventilation, the HR was 0.36 (95% CI: (0.13, 0.94). However, the interpretation of the clinical relevance of a HR is not directly possible as it is a relative effect. Its size is unexpectedly large considering the crude event probability and the Kaplan Meier-curves, as the HR often corresponds approximately to the relative risk. The discrepancy may be explained by the adjustment, differential drop-out and/or the time-dependency of the effect but a careful analysis of the cause is needed for a better understanding of the analysis and the reliability of the conclusions from this analysis.

The Kaplan-Meier curve shows separation of the survival curves only after 52 weeks. Consequently, the Cox regression model restricted to a maximal follow-up of 52 weeks shows a HR 0.87, 95% CI (0.21, 3.58). Thus, the treatment effect in terms of HR is mainly driven by the time period when the number of patients at risk was small (and constantly decreasing). Censoring appears to be differential between the treatment groups with e.g. a larger proportion of patients in the delayed start group being censored between week 52 and 78; informative censoring cannot be excluded. This increases the concerns on robustness. Adjustment for NfL has a major influence on the estimate of the HR from the Cox model.

Considering the small number of events such that single patients may have a relevant influence on the results, no firm conclusions can be drawn with respect to beneficial effects on survival.

Participant-Reported Quality of Life

According to the applicant the effects observed across exploratory participant-reported QoL measures, including ALSAQ-5, FSS, and EQ-5D, further substantiate the effects observed on the objective clinical outcome measures.

It appears that all PROS are showing continuous improvement from through week 28 to week 52. However, as already mentioned, these pooled blinded and uncontrolled data could have been considered supportive, in case the primary analysis had shown efficacy.

2.6.5.6.2. Pooling of efficacy data - 3rd Interim analysis (data cutoff 28 February 2023): Patients with long time exposure up to week 104

Study populations

The analysis results presented below, pertains to data collected during 233AS101 Part A, B and C, and the open label extension 233AS102, referred to as respective 101 Part C and 102 OLE.

The study populations reflected in the 28 February 2023 data cut were as follows:

- Pooled group CL as the full ITT population (N = 108), including:
  - Early-start participants who started tofersen 100 mg in Study 101 Part C and had the opportunity to continue on tofersen 100 mg in Study 102 (N = 72)
  - Placebo/delayed-start participants who received placebo in Study 101 Part C and had the opportunity to start tofersen 100 mg in Study 102 (N = 36)
- Part A and Part B participants who received at least 1 dose of tofersen 100 mg (either in Part B or Study 102 [N = 43] for survival analyses or in Study 102 [N = 40 overall or N = 31 for long-term tofersen exposure] for clinical function and strength,

Ninety-five of 108 participants (88%) randomized in Study 101 Part C went on to receive tofersen 100 mg in Study 102. At the time of the 28 February 2023 interim data cut, 16 participants (44.4%) in the placebo/delayed-start group and 44 participants (61.1%) in the early-start group remained ongoing in Study 102.

Figure 19: Flow of participants from Study 101 Part C to Study 102 week 104

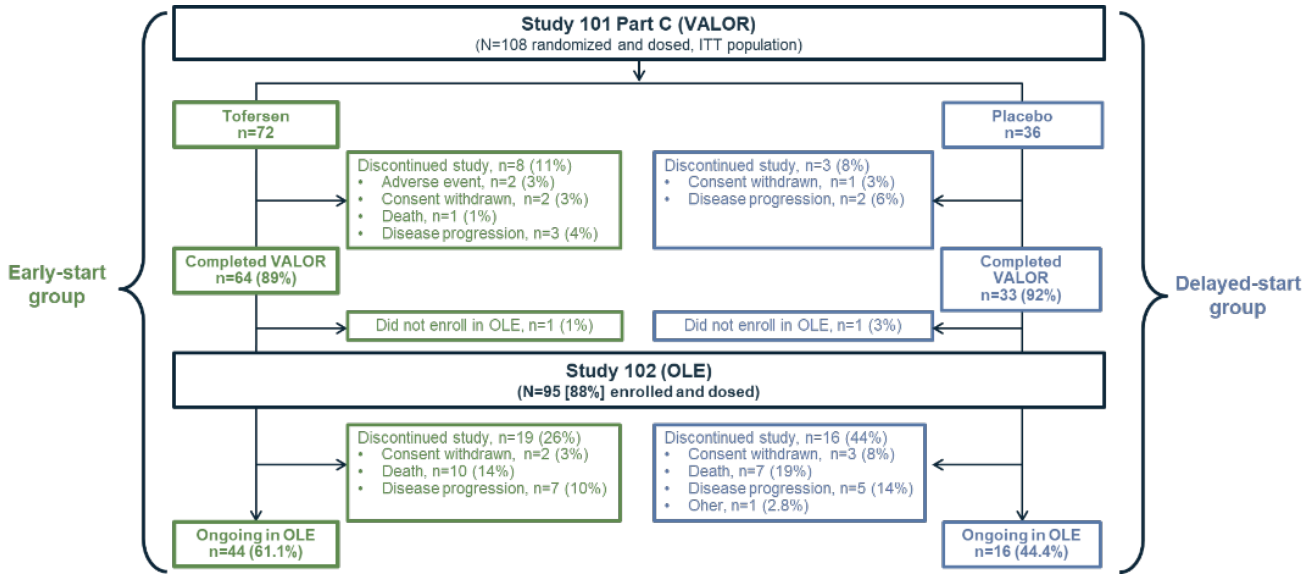


Table 22: 233AS101 and 233AS102 ISE: subject accountability for pooled group CL by Week 104 - ITT population (data cutoff 28 February 2023)

	CL ITT: 233AS101 Part C and 233AS102 (Part C subjects)	
	placebo + delayed-start tofersen 100 mg (N=36)	Early-start tofersen 100 mg (N=72)
Number (%) of subjects	36 (100)	72 (100)
Dosed in 233AS101	36 (100)	72 (100)
Dosed in 233AS102	32 (89)	63 (88)
Completed 233AS101 but not enrolled in 233AS102	1 (2.8)	1 (1.4)
Ongoing in 233AS102 at interim	16 (44.4)	44 (61.1)
Length of follow-up since 233AS101 baseline (a)		
>= 28 weeks	33 (91.7)	65 (90.3)
>= 52 weeks	28 (77.8)	59 (81.9)
>= 76 weeks	21 (58.3)	54 (75.0)
>= 100 weeks	20 (55.6)	51 (70.8)
>= 124 weeks	13 (36.1)	44 (61.1)
>= 148 weeks	10 (27.8)	37 (51.4)

(a) Counts under the length of follow-up since 233AS101 baseline are based on actual observed data of the subjects up to their last assessment in the study. Subjects who died, withdrew or did not roll over to the extension study 233AS102 before the given threshold will be excluded from the counts.

The discontinuations and the missing data play an important role in the interpretation of data. The applicant has provided subject accountability for CL-ITT population at weeks 104 and 148 and for ABL population at week 168:

Table 23: 233AS101 and 233AS102 ISE: subject accountability for pooled group CL by week 104 - ITT population

	CL ITT: 233AS101 Part C and 233AS102 (Part C subjects)	
	placebo + delayed-start tofersen 100 mg (N=36)	**Early-start tofersen 100 mg (N=72)**
Discontinued treatment prior to Week 104(a)	18 (50.0)	22 (30.6)
Adverse event	N	N
Lost to follow-up	0	0
Consent withdrawn	4 (11.1)	3 (4.2)
Investigator decision	0	0
Death	5 (13.9)	8 (11.1)
Non-compliance with study protocol	0	0
Pregnancy	0	0
Disease progression	7 (19.4)	9 (12.5)
Other	N	N
Withdrew from study prior to Week 104	15 (41.7)	21 (29.2)
Adverse event	N	N
Lost to follow-up	0	0
Consent withdrawn	3 (8.3)	3 (4.2)
Investigator decision	0	0
Death	5 (13.9)	8 (11.1)
Non-compliance with study protocol	0	0
Pregnancy	0	0
Disease progression	7 (19.4)	8 (11.1)
Other	0	0

(a) Defined as subjects who did not complete the study treatment and received the last study drug administration prior to Week 104. N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

Forty-four of 68 participants (65%) from Study 101 Part A and B went on to receive tofersen in Study 102. At the time of the 28 February 2023 interim data cut, 28 of 68 participants (41.2%) remained ongoing in Study 102. A total of 43 participants (63.2%) received at least 1 dose of tofersen 100 mg in Study 101 Part B or Study 102, and 40 participants (58.8%) received at least 1 dose of tofersen 100 mg

in Study 102, comprising the OLE tofersen 100 mg group. A total of 31 (45.6%) participants comprises the "ABL long-term tofersen 100 mg population".

Table 24: 233AS101 and 233AS102 ISE: subject accountability for pooled group CL by week 148 - ITT population

	CL ITT: 233AS101 Part C and 233AS102 (Part C subjects)	
	placebo + delayed-start tofersen 100 mg (N=36)	**Early-start tofersen 100 mg (N=72)**
Discontinued treatment prior to Week 148(a)	20 (55.6)	25 (34.7)
Adverse event	N	N
Lost to follow-up	0	0
Consent withdrawn	5 (13.9)	4 (5.6)
Investigator decision	0	0
Death	**6 (16.7)**	**9 (12.5)**
Non-compliance with study protocol	0	0
Pregnancy	0	0
Disease progression	7 (19.4)	9 (12.5)
Other	N	N
Withdrew from study prior to Week 148	19 (52.8)	24 (33.3)
Adverse event	N	N
Lost to follow-up	0	0
Consent withdrawn	4 (11.1)	4 (5.6)
Investigator decision	0	0
Death	**7 (19.4)**	**9 (12.5)**
Non-compliance with study protocol	0	0
Pregnancy	0	0
Disease progression	7 (19.4)	9 (12.5)
Other	N	N

(a) Defined as subjects who did not complete the study treatment and received the last study drug administration prior to Week 148
N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

In the CL-ITT population the percentages of deaths in those who discontinued treatment or withdrew from the study was smaller in the early start (12.5% and 12.5%) compared to the placebo/delayed start tofersen group (16.7% and 19.4%, respectively) at Week 148.

Table 25: 233AS101 and 233AS102 ISE: Subject accountability for pooled group ABL by Week 168 - ITT population

	ABL overall ITT: 233AS101 Part A, B and 233AS102 (Part A and B subjects)
	All doses (N=68)
Discontinued treatment prior to Week 168(a)	12 (17.6)
Adverse event	1 (1.5)
Lost to follow-up	0
Consent withdrawn	2 (2.9)
Investigator decision	0
Death	3 (4.4)
Non-compliance with study protocol	0
Pregnancy	0
Disease progression	5 (7.4)
Other	1 (1.5)
Withdrew from study prior to Week 168	15 (2.1)
Adverse event	0
Lost to follow-up	1 (1.5)
Consent withdrawn	4 (5.9)
Investigator decision	0
Death	5 (7.4)
Non-compliance with study protocol	0
Pregnancy	0

	ABL overall ITT: 233AS101 Part A, B and 233AS102 (Part A and B subjects)
	All doses (N=68)
Disease progression	4 (5.9)
Other	1 (1.5)

NOTE 1: Four subjects completed treatment during 233AS101 but did not complete the study. These subjects are included under withdrawals from the study. (a) Defined as subjects who did not complete the study treatment and received the last study drug administration prior to Week 168.

The table below shows that there is large heterogeneity even in the small percentage of ALS patients (~2%) who have the SOD1 mutation (approximately 1,000 prevalent cases in the European Union currently living with SOD1-ALS). The second observation one can make is that not all the patients who are ongoing in the study have the "good variants" or "long survivor variants" (e.g. p.Ile114Thr), which could have been responsible for long term survival and minimal changes in their function and condition. Except for very selected variants little is known about variability in the disease course. Overall, there is no indication that distribution of variants is unequal between groups, which may have biased the results. A5V (p.Ala5Val) is one of the most well studied variants with a median disease duration of ~1.0 to 1.2 years. With 3 A5V carriers ongoing as of the data cutoff, the median duration from ALS symptom onset to death or censoring was 1.9 years (range: 0.9 to 4.8 years) in the early-start group and 1.3 years (range: 0.7 to 2.9) in the delayed-start group.

Table 26: Subject accountability of ITT population with data cutoff 28 February 2023 (>=148 weeks follow-up)

Variant	N	CL1= Early / CL2= Delayed start tofersen	On going	Censored reason Death, PV or Disease progression	Other
p.Ile114Thr	20	N	N	N	N
p.Ala5Val	17	N	N	N	N
p.Gly94Cys	6	N	N	N	N
p.His47Arg	5	N	N	N	N
p.Leu145Phe	4	N	N	N	N
p.Ala90Val	4	N	N	N	N
p.Gly38Arg	4	N	N	N	N
p.Glu101Gly	3	N	N	N	N
p.Gly94Ser	3	N	N	N	N
p.Leu85Phe	3	N	N	N	N
p.Arg116Gly	2	N	N	N	N
p.Asp91Ala	2	N	N	N	N
p.Leu39Val	2	N	N	N	N
p.Phe65Leu	2	N	N	N	N
p.Thr138Ile	2	N	N	N	N
p.Val149Gly	2	N	N	N	N
p.Ala141Gly	1	N	N	N	N
p.Ile113Thr	1	N	N	N	N

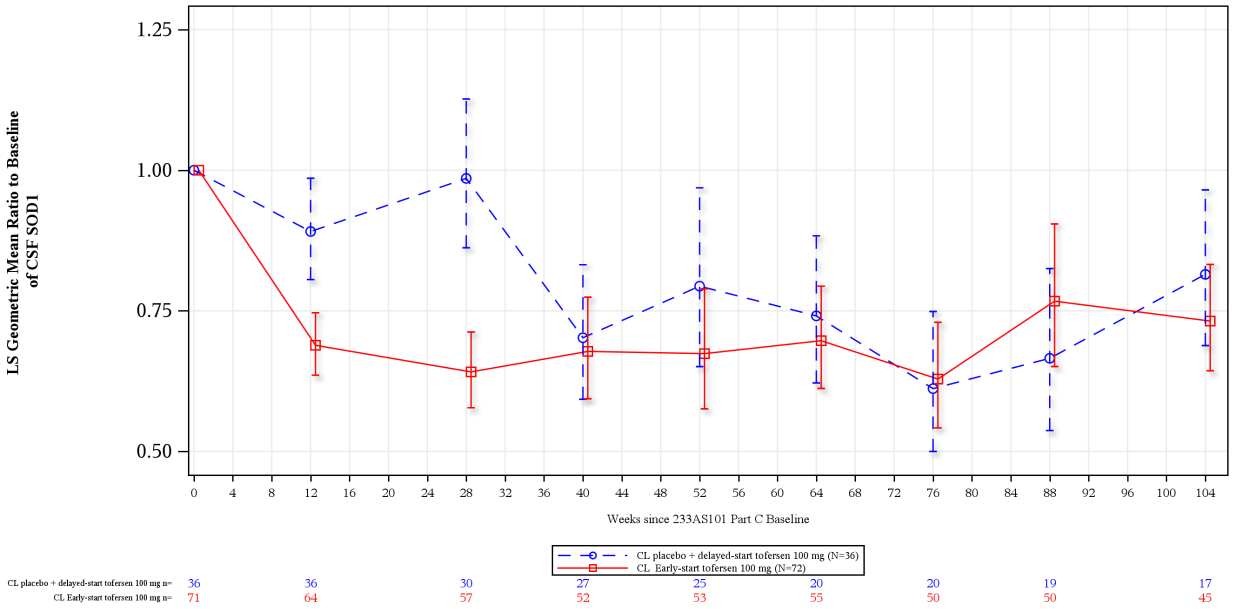
N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

Biomarkers: SOD1 and NfL up to week 104 (data cutoff 28 February 2023)

Based on the knowledge gathered up to now, a mutation in the SOD1 gene is responsible for the accumulation of toxic SOD1 protein, which consequently drives loss of motor neurons, leading to progressive loss of muscle mass, strength, function, and ultimately death. SOD1-ALS is a progressive disease with fewer than 1,000 patients in EU (ultra-rare disease).

Tofersen reduces the production and accumulation of SOD1 causative protein, as it was demonstrated in Study 101 Part C (placebo-controlled data) and its open-label extension (OLE or LTE), Study 102.

Figure 20: 233AS101 and 233AS102 ISE: line plot of total CSF SOD1 protein LS geometric mean ratio to baseline values (95% CI) by time point (up to week 104) from ANCOVA analysis using MI - ITT population



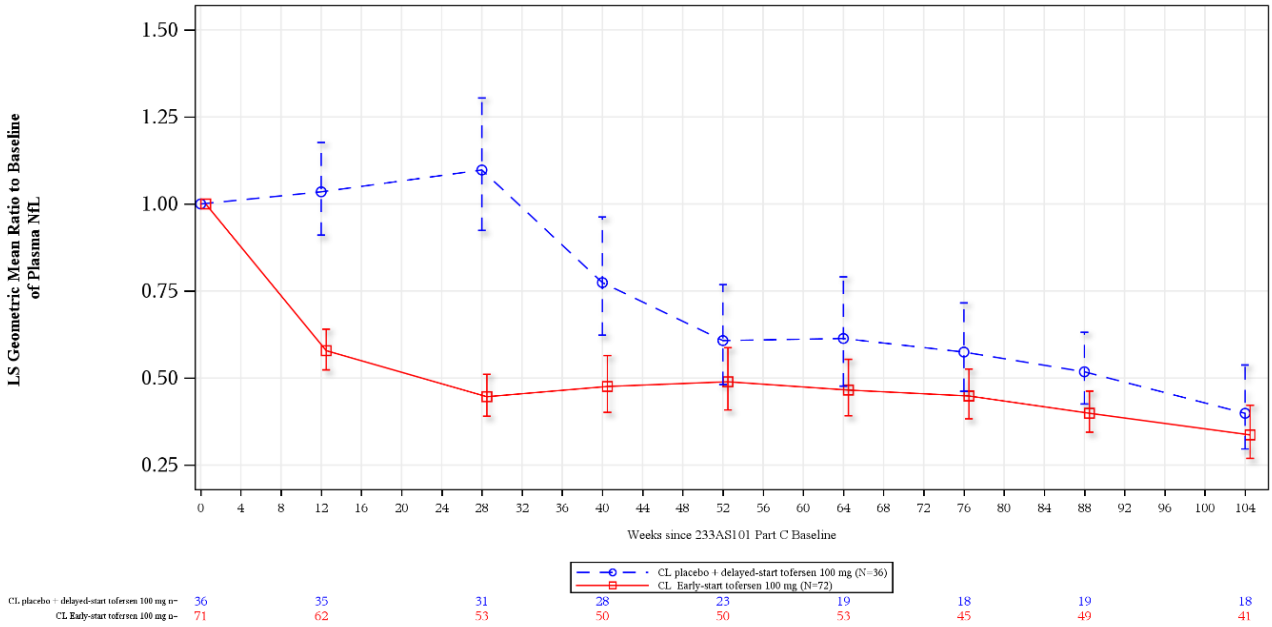
NOTE 1: Baseline is defined as day 1 value prior to the study drug. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Lower limit of quantitation (LLOQ) is 15.6 ng/mL. Values below limit of quantitation (BLQ) are set to half of LLOQ in calculations.
NOTE 3: Multiple imputation including treatment group, use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data.
NOTE 4: The analysis is based on ANCOVA model with natural log transformed data. The model includes covariates for the corresponding baseline value i.e. log value, and use of riluzole or edaravone.
Abbreviations: CSF = cerebrospinal fluid; SOD1 = superoxide dismutase 1; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square.

It is observed that the reduction of SOD1 in the case of the early start tofersen treatment group achieves a noticeable decrease at week 12 and reaches the lowest point at week 28, after which it remains at decreased levels until week 104. In the case of the delayed start tofersen treatment group the levels until week 28 remain high and close to the baseline levels. After week 28 there is a decrease until week 40 and then SOD1 levels for this delayed start group remain at approximately the same low levels as in the early-start group.

It can be expected that reduction of the causative protein should lead to a reduction in neuronal axonal injury and neurodegeneration, as observed by lowering of the NfL in plasma and CSF. Despite that the prognostic and predictive value of NfL in ALS is not fully established yet, NfL is being used in most of the clinical trials in ALS and it is currently recognised as a reliable biomarker for neurodegeneration in ALS.

Reductions in plasma (*Figure 21*) and CSF NfL (were sustained over time, with reductions from baseline of ~60% to 70% observed at Week 104.

Figure 21: Plasma NfL adjusted geometric mean ratio to Study 101 Part C baseline (95% confidence interval [CI]) by visit to week 104 from ANCOVA using multiple imputation (MI) – ITT population

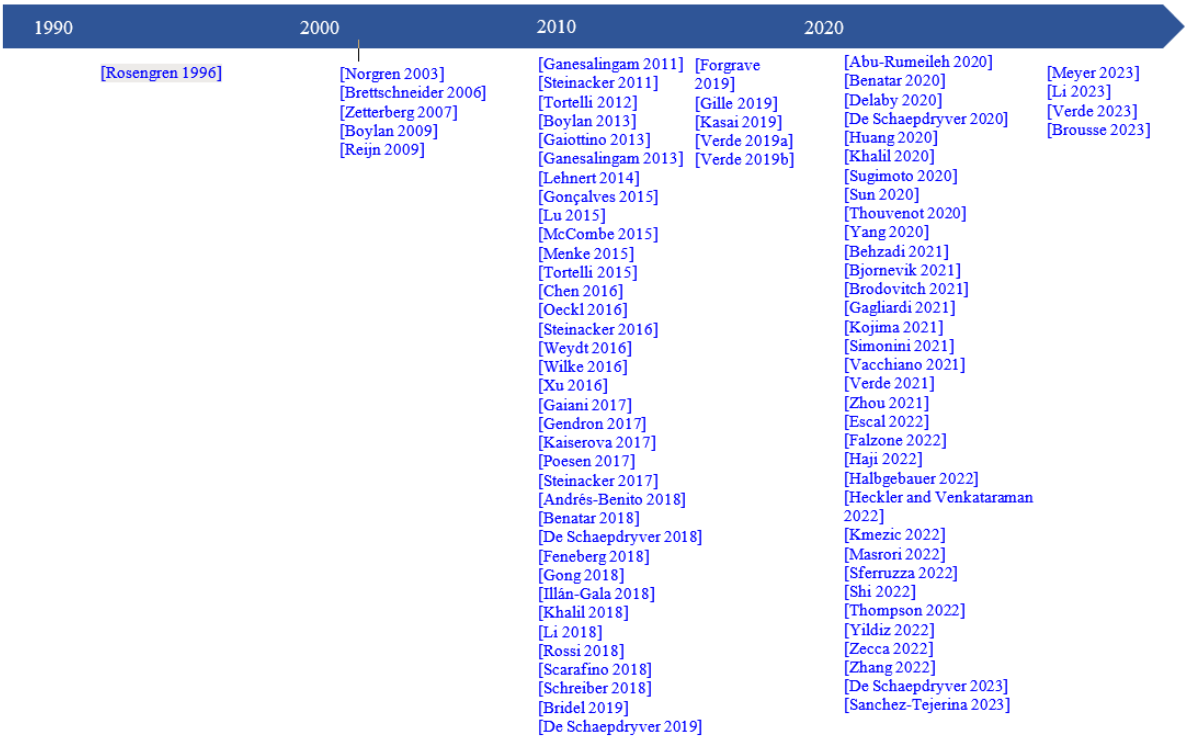


Levels of NfL reached their nadir by approximately 16 weeks after tofersen administration. The graphical pattern of the levels of NfL is quite similar to the graphical pattern of SOD1 with a time delay.

Table 27: Part of summary of secondary and exploratory endpoints at week 28

Key Endpoints	**Analysis Population**	**Placebo**	**Tofersen**	**Absolute Difference**
CSF SOD1 Protein *% change from baseline in geometric mean*	FPS (mutation/slope)	16% increase	29% reduction	45% ($p < 0.00010^a$; ANCOVA)
	SPS (mutation/slope)	19% reduction	40% reduction	21% ($p = 0.0007^a$; ANCOVA)
Plasma NfL *% change from baseline in geometric mean*	FPS (mutation/slope)	20% increase	60% reduction	80% ($p < 0.0001^a$; ANCOVA)

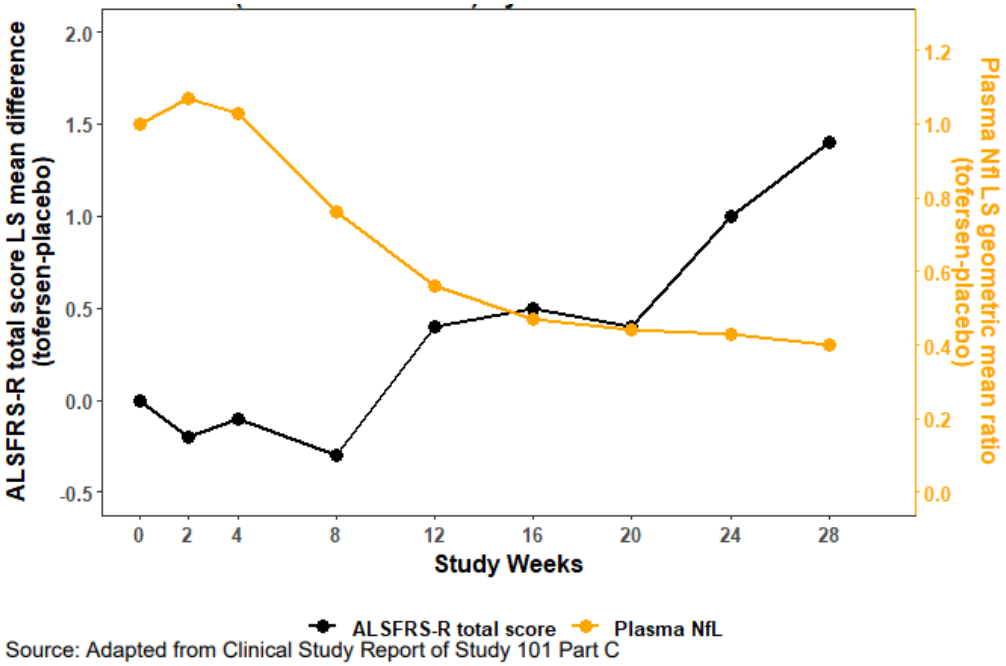
Figure 22: Timeline of neurofilament behaviour characterisation in ALS literature



According to the applicant, the relationship between the reduction in NfL and clinical function cannot be adequately assessed using correlation coefficients (CC) without adjustment for baseline NfL, as this approach does not take into account variable natural disease progression across participants. Partial correlation coefficients adjusted for baseline NfL help demonstrate that as the neurofilament levels are reduced at Weeks 12 and 16, tofersen-treated participants begin to show stabilisation at Week 28 with either no change or a smaller decline in ALSFRS-R functional score (Pearson's partial CC = -0.32; Spearman's partial CC = -0.22 for NfL reduction at Week 16 versus change in ALSFRS-R at Week 28). This relationship is strengthened when looking at reductions in NfL at Week 28 versus changes in ALSFRS-R at Week 52 (Pearson's partial CC = -0.63 and Spearman's partial CC = -0.34 in the early-start group) as participants are stabilizing and even seeing improvement in clinical function.

With tofersen treatment, NfL reductions are observable as early as 8 weeks and appear to be maximized by approximately 16 weeks after tofersen initiation. These reductions in NfL preceded discernible differences on ALSFRS-R and other clinical outcome measures, highlighting the time needed for a slowing of neurodegeneration to translate into detectable slowing of clinical decline (Figure 23).

Figure 23: ALSFRS-R total score adjusted mean difference (tofersen-placebo) and plasma NfL adjusted mean ratio to baseline (tofersen-placebo) by visit up to week 28 in Study 101 Part C



Source: Adapted from Clinical Study Report of Study 101 Part C
Note: For least squares mean calculation on ALSFRS-R total score, treatment is included as a fixed effect after adjusting for baseline disease duration since symptom onset, baseline ALSFRS-R total score, and use of riluzole or edaravone.
For least squares mean calculation on NfL, treatment is included as a fixed effect after adjusting for baseline disease duration since symptom onset, log baseline NfL, and use of riluzole or edaravone.
Abbreviations: ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale; LS, least squares; NfL, neurofilament light chain

Source: United States Food and Drug administration, Summary Basis for Approval Integrated Review Document.

ALSFRS-R and other endpoints

There is a continued separation between the placebo/delayed-start participants and the early-start tofersen treatment group over the longer-term in the ITT population adjusted for baseline plasma NfL through Week 104 (initially observed in the second interim data cut 16 January 2022, week 52.

Table 28: Change in ALSFRS-R total score from Study 101 Part C baseline at weeks, 28, 40, 76 and week 104 - ITT population adjusted for baseline plasma nfl

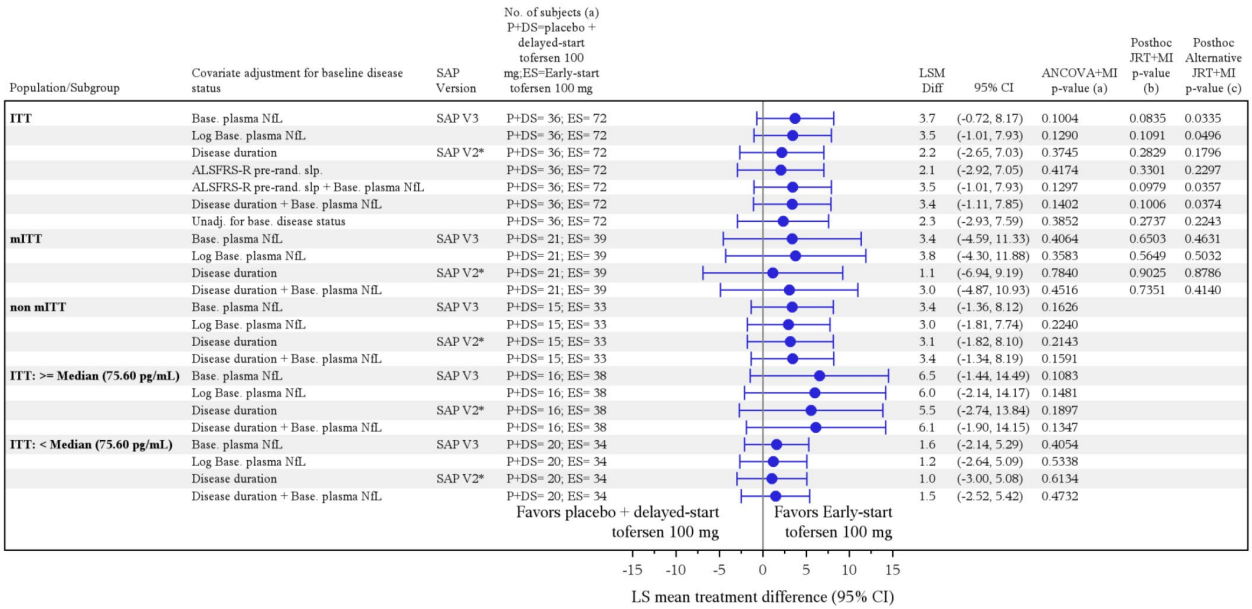
ALSFRS-R	Tofersen (n=72) vs. placebo (n=36) Change from baseline to Week 28	ISE Based on Jul 2021 Data Cut Early-start tofersen (n=72) vs. placebo → delayed start tofersen (n=36) Change from baseline to Week 40	ISE Based on Jan 2022 Data Cut Early-start tofersen (n=72) vs. placebo → delayed start tofersen (n=36) Change from baseline to Week 52	ISE Based on Feb 2023 Data Cut Early-Start Tofersen (n=72) vs. Placebo/ Delayed-Start Tofersen (n=36) Change from Baseline to Week 76	ISE Based on Feb 2023 Data Cut Early-Start Tofersen (n=72) vs. Placebo/ Delayed-Start Tofersen (n=36) Change from Baseline to Week 104
Original Multiple Imputation					
Adjusted Means: Tof; Pcb	**-4.1**; -6.2	**-5.8**; -8.5	**-6.0**; -9.5	**-7.6**; -11.0	**-9.5**; -13.2
Tof-Pcb: Adjusted Mean Difference (95% CI)	**2.1** (-0.3, 4.5)	**2.6** (-0.4, 5.7)	**3.5** (0.4, 6.7)	**3.4** (-0.3, 7.1)	**3.7** (-0.7, 8.2)
p-Value (ANCOVA+MI)	0.0904	N/A	0.0272	0.0686	0.1004

ALSFRS-R	Tofersen (n=72) vs. placebo (n=36) Change from baseline to Week 28	ISE Based on Jul 2021 Data Cut Early-start tofersen (n=72) vs. placebo → delayed start tofersen (n=36) Change from baseline to Week 40	ISE Based on Jan 2022 Data Cut Early-start tofersen (n=72) vs. placebo → delayed start tofersen (n=36) Change from baseline to Week 52	ISE Based on Feb 2023 Data Cut Early-Start Tofersen (n=72) vs. Placebo/ Delayed-Start Tofersen (n=36) Change from Baseline to Week 76	ISE Based on Feb 2023 Data Cut Early-Start Tofersen (n=72) vs. Placebo/ Delayed-Start Tofersen (n=36) Change from Baseline to Week 104
p-Value (original JRT+MI)	0.5015	N/A	0.2090	0.0860	0.0835
p-Value (alter. JRT+MI)	0.0595	N/A	0.0340	0.0375	0.0335
Multiple Imputation and Imputation Zero After Death					
Adjusted Means: Tof; Pcb				-9.0; -14.5	-10.6; -16.2
Tof-Pcb: Adjusted Mean Difference (95% CI)				**5.6** (1.0,10.1)	**5.6** (0.6,10.5)
p-Value (ANCOVA+MI)				p=0.0160	p=0.0280

The table above shows that the largest difference between early start and placebo/delayed start tofersen is observed at week 52 and then the difference between the two groups remains the same or very similar over a period of another year, which is consistent with a disease modifying effect.

The trends observed at Week 52 for Study 101 Part C, were consistently in favor of tofersen across different populations and disease progression subgroups (mITT vs. non-mITT and NfL-based), regardless of which covariates were adjusted for. This consistency favouring tofersen treatment was observed in the forest plot at week 104.

Figure 24: Forest Plot of ALSFRS-R Total Score Change From Baseline to Week 104



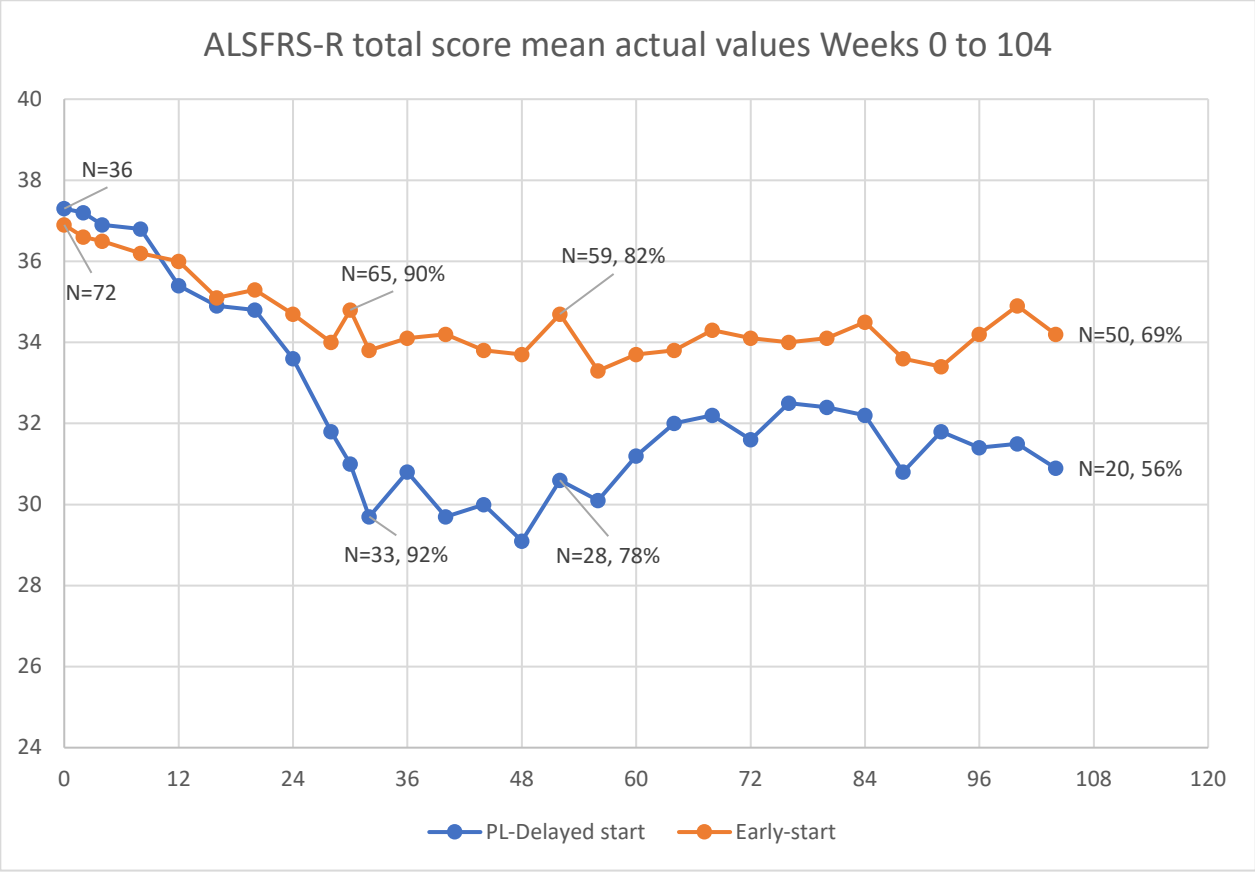
NOTE 1: SAP V2.0 is the integrated efficacy SAP that was finalized prior to the final database lock for 233AS101 and interim lock for 233AS102 based on 16 July 2021 data cut; prespecified analyses were primarily based on disease progression subgroups and adjusted for disease duration since symptom onset. SAP V3.0 was an amendment to the integrated efficacy SAP following the initial data readout and incorporated plasma NfL as a covariate with the focus on the ITT population. SAP V3.0 was finalized prior the interim lock for 233AS102 based on 16 January 2022 data cut.

NOTE 2: For ITT population, multiple imputation including the specified covariate(s), use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include the specified covariate(s), baseline ALSFRS-R, and use of riluzole or edaravone.
NOTE 3: For the analyses including disease duration since symptom onset in mITT and non mITT population, multiple imputation including use of riluzole or edaravone and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include covariates for baseline disease duration since symptom onset, baseline ALSFRS-R and use of riluzole or edaravone. For the other analyses in mITT and non mITT population, multiple imputation including baseline plasma NfL, use of riluzole or edaravone and the relevant baseline and postbaseline values for the endpoint is used for missing data. The corresponding ANCOVA models include covariates for the specified covariate(s), baseline plasma NfL, baseline ALSFRS-R and use of riluzole or edaravone.
* No statistical testing was prespecified in SAP Version 2; all p-values are post-hoc for SAP V2 analyses.
(a) From the listed ANCOVA analysis based on change from baseline.
(b) From the ANCOVA on ranked scores; deaths are ranked the lowest; MI is used for handling missing data due to withdrawals other than death.
(c) From the ANCOVA on change of ranked scores pre- and post- treatment; deaths are ranked the lowest; MI is used for handling missing data due to withdrawals other than death.
Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised; ANCOVA = analysis of covariance; MI = multiple imputation; LS = least square; P+DS = placebo + delayed-start tofersen 100 mg; ES = Early-start tofersen 100 mg.

In order to view the functional condition of the patients, crude plots were constructed using the actual observed mean values of ALSFRS-R total score (without adjustment) over time.

Measurements for ALSFRS-R at week 104 were recorded for 20 (55.6%) patients of the placebo/ delayed start tofersen treatment and for 50 (69.4%) patients from the early start tofersen group. It was considered appropriate to look at the discontinuations from the study trying to understand better the potential stabilisation of patients.

Figure 25: ALSFRS-R total score mean actual values by visit for pooled group CL (observed data) - ITT population



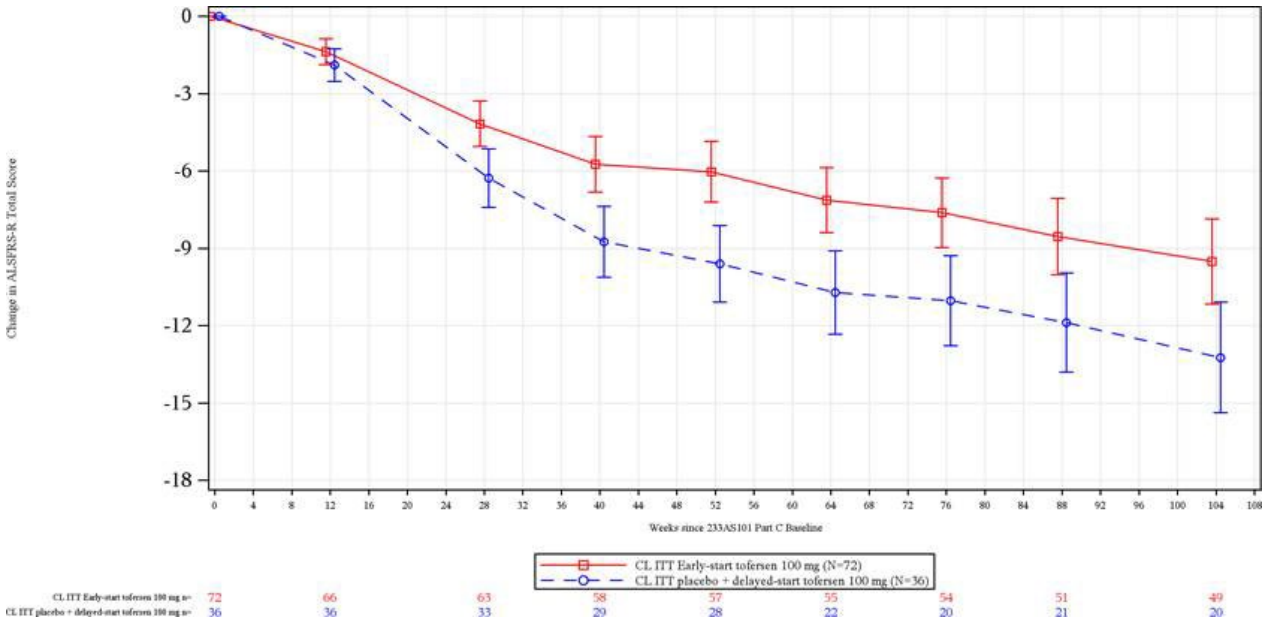
CL ITT: 233AS101 Part C and 233AS102
(At baseline CL1 N=72 and CL2 N=36, at week 104 CL1 N=50 and CL2 N=20)

Visual observation of this "crude" figure shows a greater decline in function of placebo treated patients until week 28 compared to tofersen treated patients. After week 28 a stabilisation of ALSFRS-R score in both groups is observed and this remains throughout week 104, but with a lower number of patients (see section of subject accountability below). It is also observed that the difference achieved at week 52 between the two groups remains the same over a long period of time, which is consistent with a disease modifying effect from tofersen treatment.

However, one could argue that this figure is based on complete cases, such that it is not clear whether the changes over time are real, or just due to selection, i.e. patients performing worst discontinuing study (most discontinuations were due to death or disease progression). For example, the increase in ALSFRS-R in the delayed start group between week 48 and week 76 is in parallel with a decrease of observed cases from 27 to 20. However, a patient selection to provide such a picture of stabilisation is not very likely in the view of the assessment team. The characteristics of the patients included do not suggest that there could be a subgroup of patients, who would be stable for 2 years (at least) without any tofersen intervention (see section of subject accountability below).

The ALSFRS-R total score over time (up to week 104), ANCOVA analysis using MI - ITT population (studies 101 part C and 102) is presented in the following figure. Data suggest that tofersen slows the decline in function. The difference between early and delayed tofersen start groups remains stable over time suggesting greater benefit with earlier treatment and a disease modifying effect. The limitation is the MAR assumption used for the analysis.

Figure 26: Line plot of ALSFRS-R total score LS mean change from baseline values +/- SE by time point from ANCOVA analysis using MI for pooled group CL - ITT population



The applicant presented an analysis of estimated proportion of participants with improvement in the efficacy endpoints. Results for ALSFRS-R are presented in Table 29. The findings do not show the actually observed proportions of patients with improvements, but proportions estimated based on a logistic regression model adjusting for covariates, which is considered a limitation as this usually means that the response is shown for a hypothetical population where e.g. continuous values such as NfL are set to their mean. Additionally, the model is based on strong assumptions such as proportional odds.

Table 29: Estimated Proportion (%) of Participants With Improvement or Stabilisation/Improvement on Clinical Function, Strength, and QoL Measures From Baseline to Week 100/104 – ITT Population Adjusted for Baseline Plasma NfL

Endpoint		**Week 100/104**	
		Placebo/delayed-start (n=36)	**Early-start (n=72)**
ALSFRS-R	Proportion of participants with **improvement**	9.3	16.0
	Proportion of participants with **stabilisation or improvement**	16.6	23.5

The applicant repeated the requested analysis, whereby responses should be provided as observed (considering death and withdrawal as failure, and replacing other missing values based on MI). The proportions of responders are slightly larger but absolute differences in response proportions between treatment groups are comparable to the analysis based on logistic regression.

Table 30: Proportion (%) of participants with improvement or stabilisation/improvement on clinical function, strength, and quality of life measures from baseline to week 100/104 (without logistic regression) – ITT population

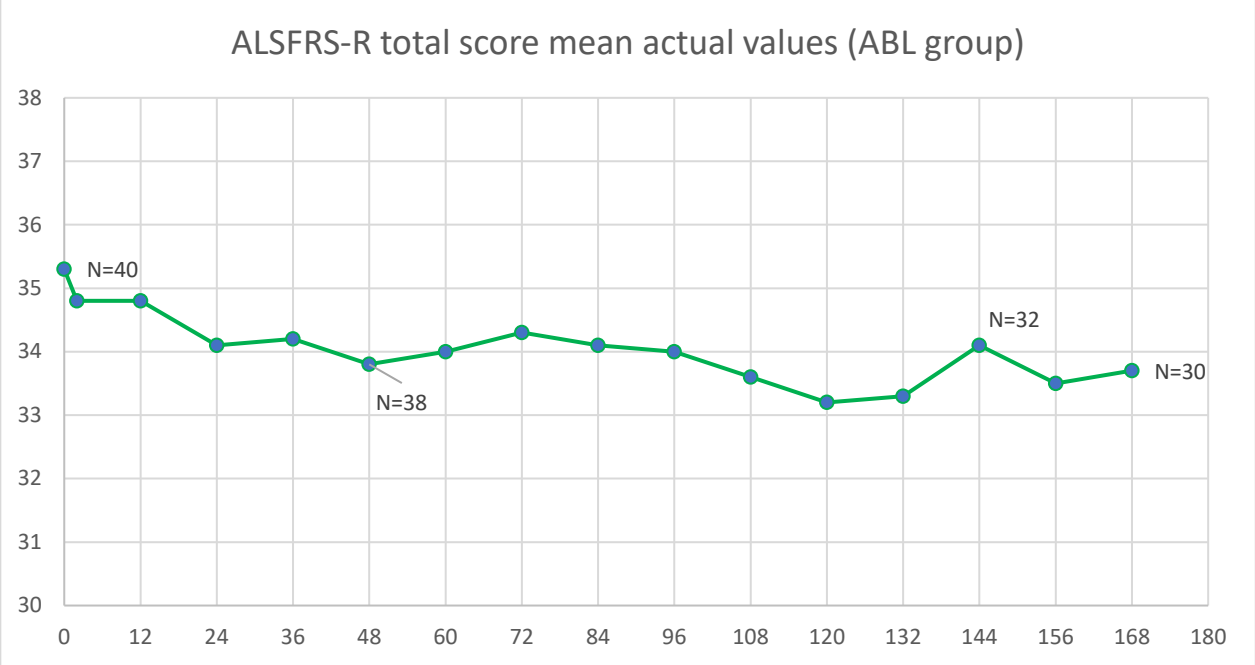
Endpoint		**Week 100/104**	
		Placebo/Delayed-Start (n=36)	**Early-Start (n=72)**
ALSFRS-R	Proportion of participants with **improvement**	**11.6**	**19.5**
	Proportion of participants with **stabilisation or improvement**	**22.7**	**29.3**
Percent-Predicted SVC	Proportion of participants with **improvement**	10.5	21.4
	Proportion of participants with **stabilisation or improvement**	10.5	21.4
HHD Megascore	Proportion of participants with **improvement**	10.1	25.8
	Proportion of participants with **stabilisation or improvement**	10.1	25.8
ALSAQ-5	Proportion of participants with **improvement**	16.1	19.7
	Proportion of participants with **stabilisation or improvement**	25.1	33.6
FSS	Proportion of participants with **improvement**	25.1	25.3
	Proportion of participants with **stabilisation or improvement**	32.8	33.6
EQ-5D-5L	Proportion of participants with **improvement**	18.2	27.7
	Proportion of participants with **stabilisation or improvement**	23.8	34.6

Note 1: Participants with any increase compared with Study 101 baseline were classed as improvement responders, and participants with no change or with a decrease compared with Study 101 baseline were classed as non-responders. Deaths and withdrawals are classed as non-responders. All other missing data are accounted for using MI.
Note 2: For ALSFRS-R, SVC, and HHD, results are presented at Week 104. For ALSAQ-5, FSS, and EQ-5D-5L, results are presented at Week 100. The difference in assessment week is due to assessments being performed at different visits.

These data in the CL-ITT population appear to be consistent with observations for Study 101 Parts A and B in which disease progression has essentially been stabilized for a median of 4.7 years (range: 3.1 to

5.8 years) of follow-up in the cohort of participants who received at least 1 dose of tofersen in Study 102 (n = 40). These participants experienced a mean change from Study 102 baseline to Week 168 (~3.2 years) of -2.3 points on ALSFRS-R, -2.4 on percent-predicted SVC, and -0.1 on HHD megascore.

Figure 27: 233AS101 and 233AS102 ISE: ALSFRS-R total score mean actual values and change from baseline by time point for pooled group ABL (Study 101 Parts A and B and study 102)



N=40 at baseline and N=30 (~75%) at week 168

A mean change from Study 102 baseline to Week 168 of -2.3 points in ALSFRS-R over 39 months (~3.2 years) could be considered an indication of stabilisation of the disease, despite that the data are uncontrolled. However, these results may derive from a very selected patient population, which may not be at all representative of the ALS-SOD1 population and are uncontrolled. These data should be interpreted with extreme caution. The applicant discussed whether these ABL patients had specific characteristics that would allow them to remain stable in their ALS condition irrespective of tofersen treatment (see discussion of Clinical Efficacy).

Despite that these data should be interpreted carefully; they may contribute to the suggestion that a potential stabilisation in tofersen-treated participants is unlikely attributable to biological/measurement noise. This is even more evident in the case of placebo/delayed-start tofersen treatment group for which potential stabilisation was shown after the initiation of tofersen treatment.

In the absence of a long-term placebo-controlled study correlation analyses between change in NfL and change in ALSFRS-R at a later time point are of interest. Under the Raw Data Pilot project, the subject profiles using change from baseline in NfL and change from baseline in ALSFRS-R showed some correlations in some patients but not in others. The graphs were provided by the Data Analysis Centre in groups of 10 patients to avoid confusion with many lines in the same area. Two of these graphs with the early and placebo/delayed start tofersen treatment groups and patients having the rapidly progressing p.Ala5Val (A5V) variant, as examples, are presented below.

Figure 28: Change from Study 101 Part C baseline ALSFRS-R total score and log(NfL) profiles, gene variant Ala5Val, early start tofersen

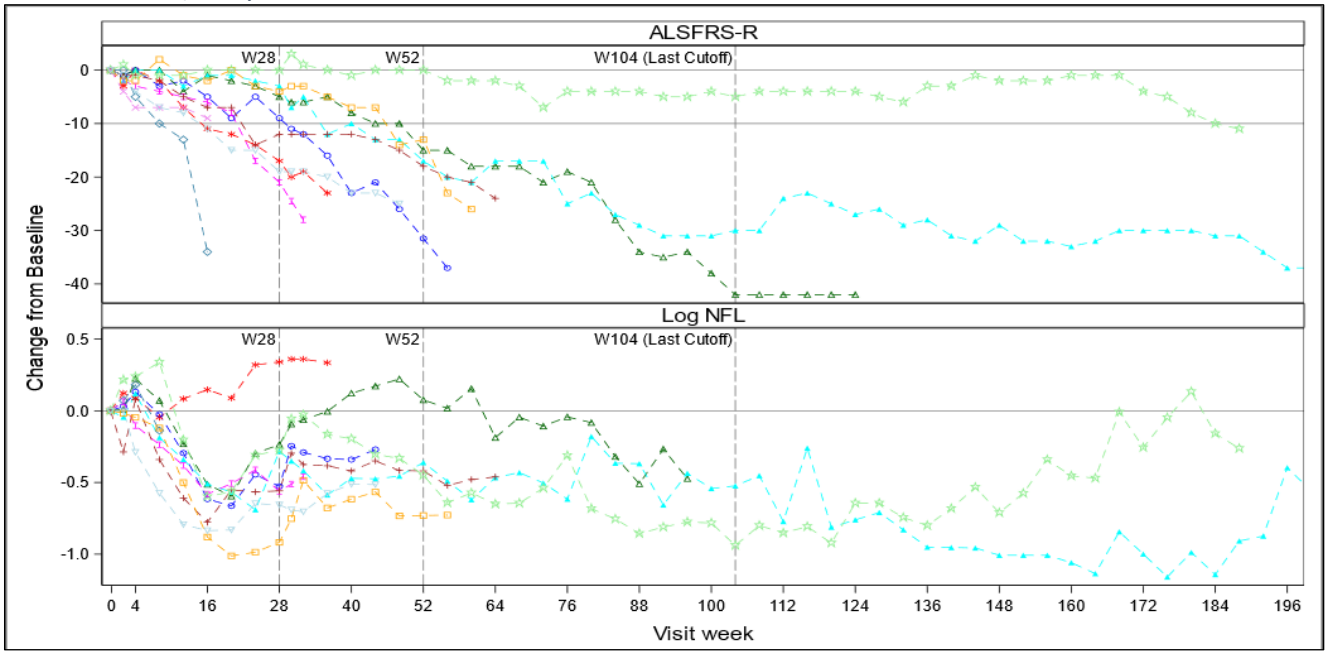
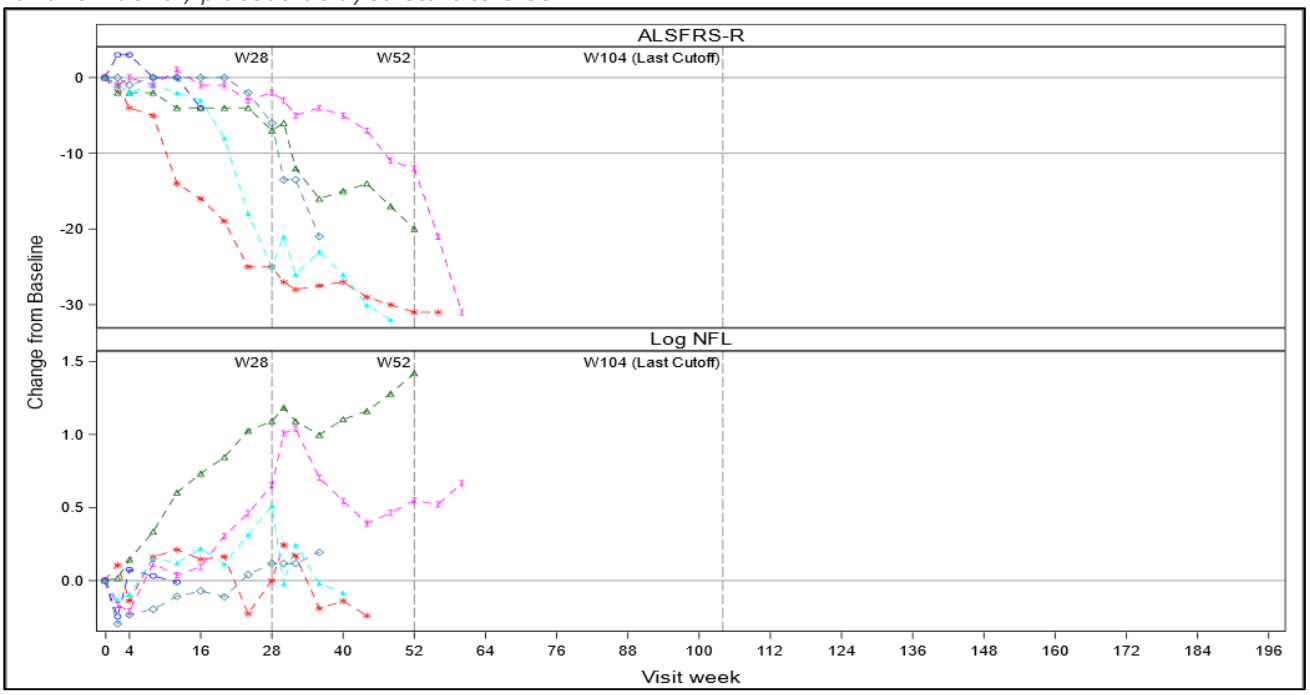


Figure 29: Change from Study 101 Part C baseline ALSFRS-R total score and log(NfL) profiles, gene variant Ala5Val, placebo delayed start tofersen



A5V variant is among the best characterized SOD1 variants and consistently found to be associated with a median disease duration of approximately 1 to 1.2 years (Bali 2017; Opie-Martin 2022). In the above graphs a clear difference between the early start and the placebo/delayed start tofersen is visually obvious, also indicating a beneficial effect of early tofersen treatment.

Event-free survival

The applicant provided analyses for three different time to event endpoints: Time to death or PV, Time to death, Time to death or PV or withdrawal due to disease progression.

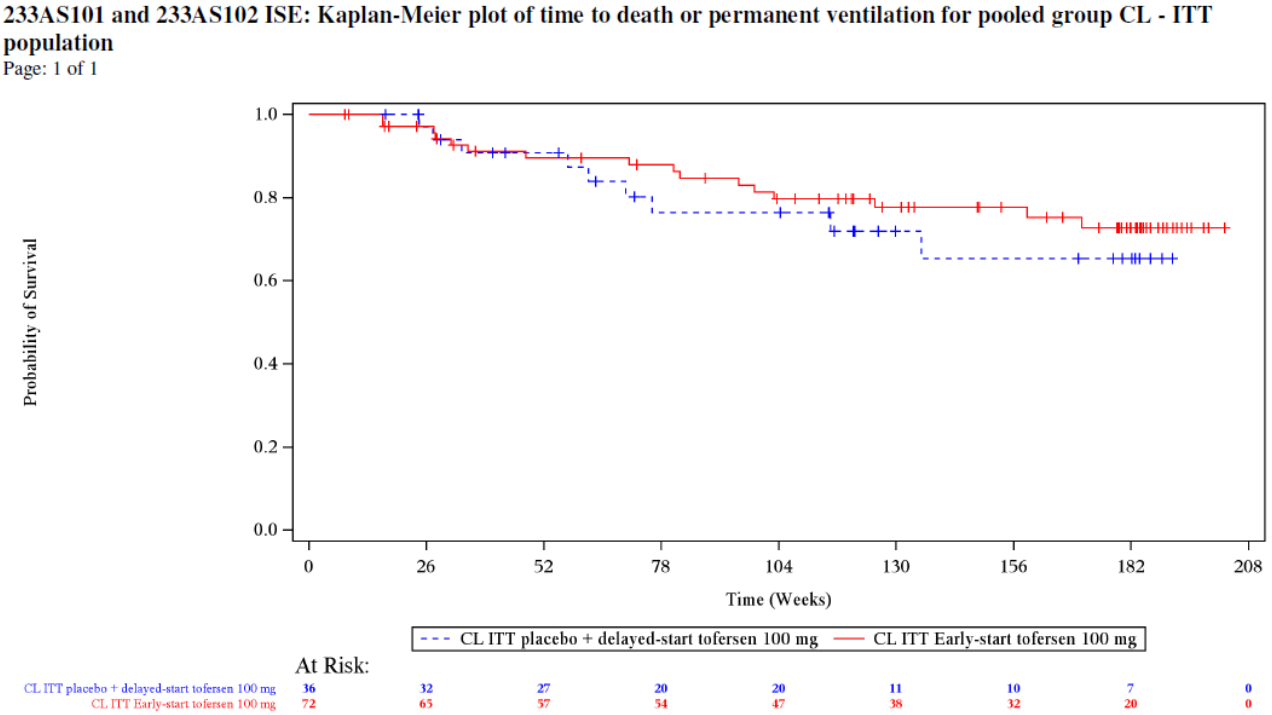
The applicant claims that all available data consistently indicate that tofersen, particularly when initiated early, has a clinically meaningful effect on event-free survival.

However, the overall number of events (deaths or PV) at latest data cut-off (28. February 2023) is small (see table below) such that the uncertainty of estimates derived from all analyses is high and no robust conclusions are possible.

Table 31: Additional time to event analyses

Description	Hazard ratio (95% CI)	Wald test	Log-rank test	Events of total in early group	Percentage, early	Events of total in delayed group	Percentage, delayed
Time to death or Permanent Ventilation (21 days)	0.76 (0.332, 1.720)	0.5049	0.4843	16/72	22.2%	9/36	25.0%
Time to death	0.66 (0.252, 1.705)	0.3870	0.3486	11/72	15.3%	7/36	19.4%
Time to death or Permanent Ventilation (21 days) or Disease Progression	0.73 (0.378, 1.422)	0.3586	0.3669	24/72	33.3%	14/36	38.9%

Figure 30: 233AS101 and 233AS102 ISE: Kaplan-Meier plot of time to death or permanent ventilation for pooled group CL – ITT population



NOTE 1: Time to death or permanent ventilation is defined as the time from first dose to death or permanent ventilation (>= 22 hours of mechanical ventilation [invasive or noninvasive] per day for >= 21 consecutive days), whichever comes first. Subjects who do not meet the endpoint definition are censored at the subject's last known alive date. Only events that were adjudicated by the Endpoint Adjudication Committee are included.
NOTE 2: + indicates censored data.

The crude event rates and Kaplan-Meier curves show only small numerical differences in favor of early start tofersen. The marginal HR's (i.e. unadjusted HR) from a Cox model without including covariates are consistent with these results, being numerically in favor of tofersen ranging from 0.66-0.76 but with wide 95% CIs.

The applicant's claim of a larger effect is based on a Cox model including baseline NfL as a covariate (see Table 32 below).

Table 32: Part of the summary of time-to-event analyses (Study 101 Part C + 102)

	Time to death or PV	**Time to death**	**Time to death, PV, or withdrawal due to disease progression**
ITT			
HR (95% CI)	**0.47** (0.20, 1.11)	**0.36** (0.13, 1.02)	**0.50** (0.25, 0.99)
Log-rank p-value[a]	0.1685	0.1224	0.0866
Cox p-value	0.0842	0.0538	0.0475

HR: hazard ratio.
[a]Based on log rank test stratified by median baseline plasma NfL.

The resulting conditional HR does not only adjust for imbalances but has a different interpretation than the marginal HR. Adjustment for NfL was included in the SAP for the pooled analysis after results from study 101 part C were known. Furthermore, the analysis is based on the proportional hazards assumption for baseline NfL (i.e. assuming a continuous increase in hazard with increasing baseline NfL) and proportional hazards over time. Both are strong assumptions, and it is questionable that the model is robust to deviations from these assumptions, also considering the small number of events such that single patients may have a relevant influence on the results.

To contextualize the survival in the trial participants with the expected natural history of *SOD1*-ALS, the applicant provided additional analyses of "disease duration" by estimating time from ALS symptom onset to death or censoring. In addition, a rank preserving structural failure time model to compare what is observed in the early-start tofersen participants with what would have been expected in the delayed-start group if they had they not switched to tofersen in Study 102. However, these analyses are based on strong and unverifiable assumptions such that the robustness of these analyses is highly questionable. Firm conclusions cannot rely on such hypothetical approaches.

Overall, although numerical trends in favour of the early start tofersen group were observed, no conclusions can be currently drawn on an effect of tofersen on time to event-free survival because robustness is highly questionable due to small event numbers and immature data and the strong assumptions made for analysis.

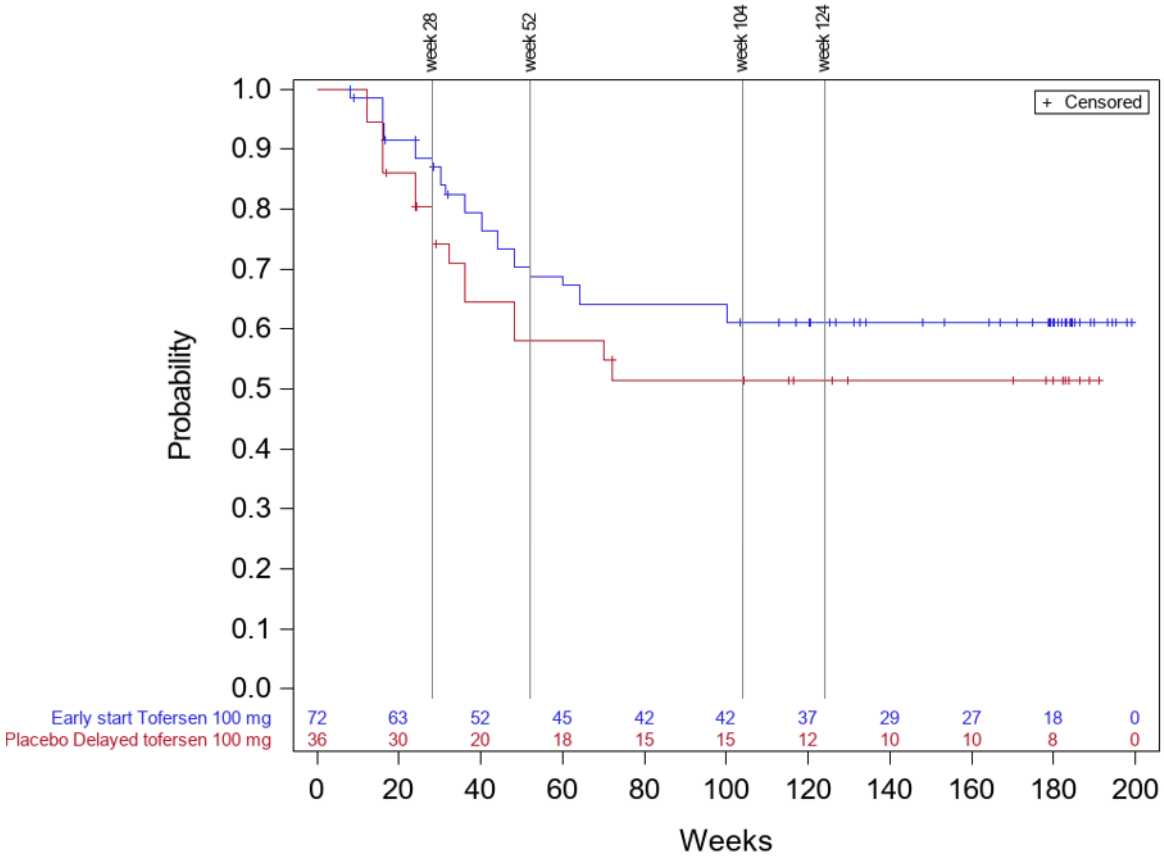
Additional analyses were also performed under the Raw Data Pilot project for the composite event: change from baseline ALSFRS <-10, death or PV assistance and a comparison was made between subjects allocated to tofersen versus in 101 Part C and observed into 102 OLE. This was performed with the data by the applicant. Two models comparing events up to week 124 (any events occurring later are censored) have been applied for the comparison, one with and one without baseline NfL included, and with a noteworthy difference to the outcome of the comparison.

Table 33: Comparison between treatment on new composite event endpoint (week 124)

Description	Hazard ratio (95% CI)	Wald test	Log-rank test	Events of total in early group	Percentage, early	Events of total in delayed group	Percentage, delayed
Time to change<-10 or death or PV	**0.70** (0.375, 1.309)	0.2646	0.2645	26/72	36.1%	16/36	44.4%
Time to change<-10 or death or PV, Adjustment for log (baseNfL)	**0.46** (0.241, 0.883)	0.0194	0.0463	26/72	36.1%	16/36	44.4%

This Table and the graph below indicate a better outcome for the patients with early tofersen treatment, especially when NfL is taken into account. However, the underlying non-informative censoring assumption is questionable, which is a limitation of this analysis as it may lead to an overestimation of the proportion of event-free patients in both groups.

Figure 31: Kaplan Meier curves for event: Time to change from baseline ALSFRS-R total deterioration greater than 10 (< -10) points, Death or Permanent ventilation assistance, whichever comes first



Cumulative distribution function analyses (week 52)

Under the Raw Data Pilot project some additional "responder like" analyses were also conducted.

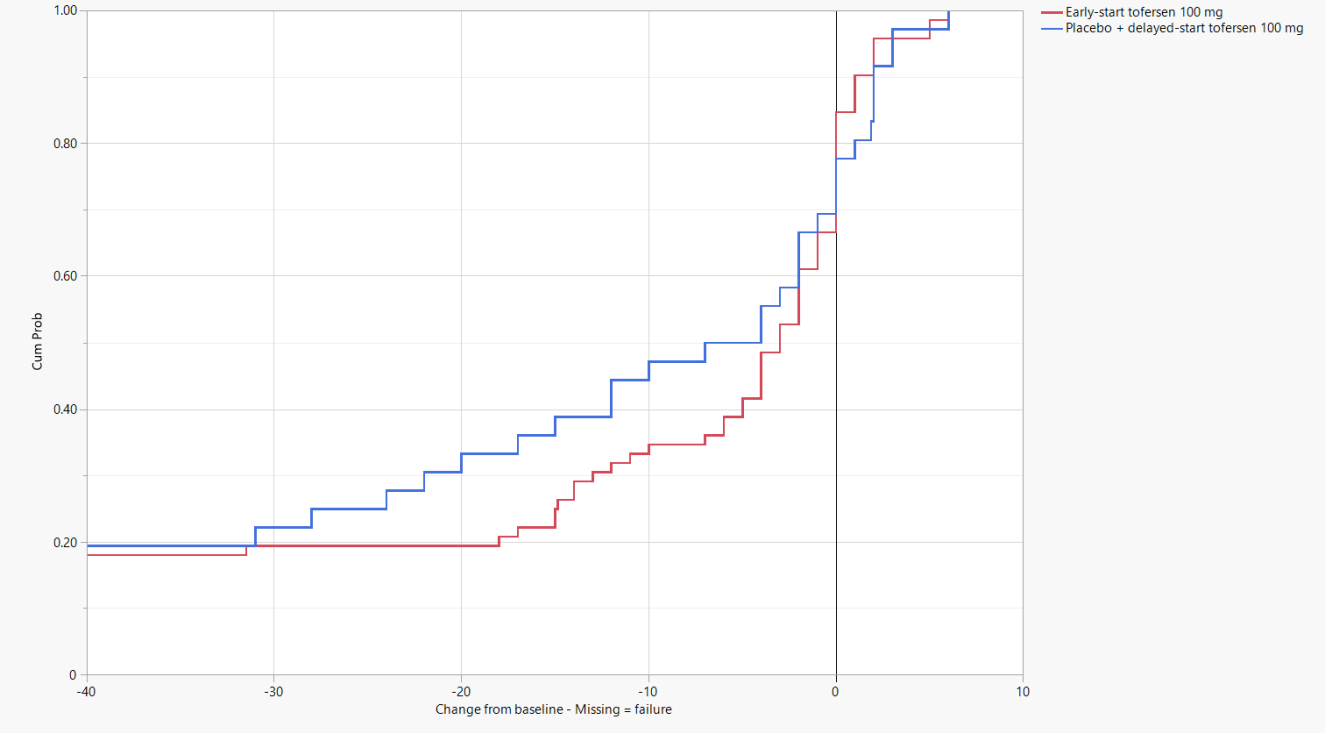
In these analyses, the cumulative distribution functions of the change from baseline in the ALSFRS-R total score were generated for both treatment groups indicating the probability of failure (y axis) projected versus change from baseline as cut-off value defining failure, taking into consideration

treatment discontinuation due to death, progressive disease, AE and other subject withdrawal as failure expressed as a decrease from baseline of -40 being lower than any other change from baseline.

For those two subjects that did not enter extension study 102, changes were extrapolated from week 28 to week 52. For one subject with intermediate missing value at week 52 the change was interpolated between week 48 and week 56.

Interpretation is that the proportion of subjects with a change lower than (and equal to) that on the x-axis is given by the value on the y-axis. Lower change means larger decrease, hence, failure. 1 – this proportion would be the response rate. In the graph below which shows, e.g., a probability of failure for a cut-off of -10, a rate of about 35% and 47% for the failure rates corresponds to early start and placebo delayed start tofersen treatment group, respectively. Values at -40 relate to the proportion of subjects that discontinue the study (except those 2 subjects that did not enter the extension study, see above).

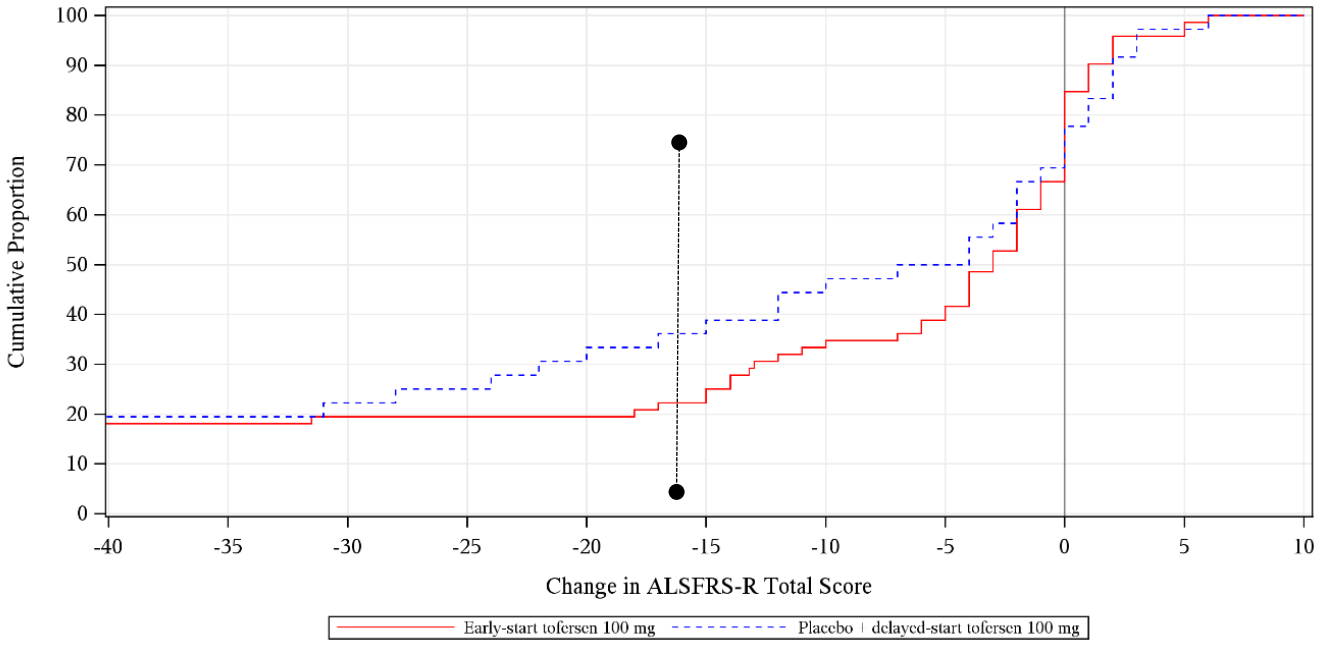
Figure 32: Cumulative distribution function of ALSFRS-R changes from baseline at week 52 indicating the probability of failure at week 52 by differing cut-off values.



The applicant has repeated a cumulative distribution function analyses and discussed the results.

In the following figure, very similar to the one generated during the Raw Data Pilot Project, for a cutoff of -12, the rates of worsening in ALSFRS-R are approximately 32% and 44% for the early-start tofersen group and the placebo/delayed-start tofersen group, respectively. These shows that participants in the placebo/delayed-start group tend to have a higher rate of worsening in ALSFRS-R at Week 52.

Figure 33: Cumulative distribution function of ALSFRS-R changes from baseline at week 52 indicating the probability of failure at week 52 - ITT population



The most reliable analysis is at week 52 with missing data of approximately 20% and only half a year without placebo data. A 6-month delay in the treatment effect for a disease modifying agent would be reasonable.

There are certainly various caveats with such analyses e.g. interpretation of missing data and unresolvable multiplicity issues (referring to *post hoc* analyses). The most reasonable cut-off (or range of cut-offs) should rather be defined independently of the graph, e.g. in terms of what can be expected as a change for this period and the intra-subject variability/fluctuation. P-value of the corresponding non-parametric test would be 0.6 (not adjusted by NfL baseline values), i.e. a significant difference between the Cumulative Distribution Function (CDFs) is not given even if this analysis had been pre-specified and difference between CDFs (hence distributions) can still be due to chance. However, the results suggest (with support from pre-clinical and biomarker/PD data) that tofersen treatment may exert a disease modifying effect.

2.6.5.7. Supportive studies

The following data are considered supportive of the main study 101 Part C and the pooled analyses together with some of the study 102 LTE data (interim 2, data cut: January 2022).

2.6.5.7.1. Expanded Access Program

An EAP providing access to tofersen outside the context of a formal clinical trial or marketing authorisation was implemented for rapidly progressing SOD1-ALS patients in July 2021. The applicant expanded eligibility to include all SOD1-ALS patients as of 17 October 2021.

Safety data from EAP participants available as of the 15 July 2022 safety data cut for this application. As the EAP is not a clinical trial, efficacy data are not routinely or consistently collected; therefore, no efficacy data from EAP are included in this application. However, a case of an EAP participant with a relatively quickly progressing SOD1 mutation was also presented. This individual experienced a

maintenance of a stable score on ALSFRS-R over 1 year of treatment and improvements on the timed up and go and 10-meter walk test.

2.6.5.7.2. Continuous Evidence Generation Plan

The applicant evaluated various approaches to generate additional comprehensive clinical data, including whether these data could be generated by a randomised, placebo-controlled clinical trial. For this, informed by the learnings from the tofersen clinical studies and the evolving ALS literature, the applicant modelled alternative trial designs to confirm the results seen to date with statistical significance.

Based on this assessment, the applicant does not believe that additional placebo-controlled studies are feasible.

The applicant plans to collect long-term efficacy and safety data via the ongoing Study 102 and Study 303 (ATLAS) as well as supplementary real-world evidence via existing disease registries. An integrated analysis of Study 101 Part C and Study 102, when all participants have had the opportunity to have at least 3.5 years of follow-up since randomisation will be reached in Q3 2024. In addition, the evidence generation plan includes variant-specific survival analyses (incorporating data from tofersen clinical trials, natural history datasets/literature, and real-world evidence collected via disease registries).

Study 101 Part C and Study 102

The open-label Study 102 LTE is currently scheduled to continue through Q3 2024 to enable up to 7 years of treatment and follow-up, with the longest follow-up from patients who enrolled into Study 102 from either Parts A or B of Study 101, and to provide important supportive long-term data on the effect of tofersen on the trajectory of the disease and overall survival. For those participants randomised in Study 101 Part C, the longest follow-up will be approximately 5 years.

Study 233AS303 (ATLAS)

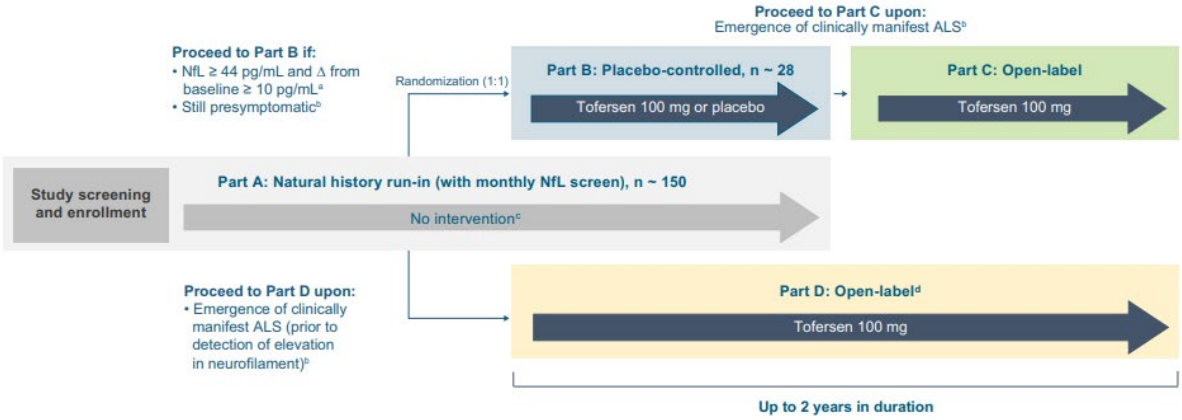
Study 233AS303 is an ongoing, phase 3, randomized, double-blind, placebo-controlled, 4-part study designed to evaluate whether tofersen, when initiated in pre-symptomatic carriers of a SOD1 mutation with biomarker evidence (increased plasma NfL) of disease activity, can halt or delay the emergence of clinically manifest ALS and/or slow the decline in function once symptoms are observed compared to the initiation of tofersen at the time of, or after, the emergence of clinically manifest ALS. The selection of the population and the design of Study 303 was informed by the presymptomatic familial ALS (pre-fALS) natural history study (NCT00317616). Pre-fALS is a prospective natural history and biomarker study of people not yet affected with ALS, but who are at genetic risk for developing ALS. Unaffected (healthy) people from familial ALS (fALS) pedigrees in which a known genetic mutation associated with ALS has been identified are recruited. For this study, a fALS pedigree is one with two biologically related individuals who have or have had ALS and/or frontotemporal dementia (FTD). Individuals who may be at genetic risk for ALS and who belong to families with at least one affected family member who has tested positive for a known ALS genetic mutation may also be eligible to participate. Data from these individuals in the pre-fALS study were used to model the presymptomatic changes in NfL and to determine the study's predefined NfL threshold for Part B. This model was then used to estimate the expected phenoconversion rate after reaching the predefined NfL threshold and hence inform the sample size and duration of Study 303.

Approximately 150 pre-symptomatic at-risk carriers of highly or completely penetrant and rapidly progressive SOD1 mutations will be enrolled in the natural history portion of the study (Part A). Individuals in Part A who experience an increase in plasma NfL that reaches the protocol-defined threshold (≥ 44 pg/mL and ≥ 10 pg/mL increase from baseline) and remain clinically pre-symptomatic will have the opportunity to enrol in the interventional, placebo-controlled study period (Part B; n = 28).

Participants in Part B who experience an emergence of clinically manifest ALS will be eligible to screen for the OLE (Part C). The duration of participation in Part B and/or Part C will be up to 2 years. Participants who experience an emergence of clinically manifest ALS prior to detection of elevation in NfL will be eligible to screen for an open-label interventional period for up to 2 years (Part D).

The population enrolled in Study 303 is different from that of Study 101 Part C but represents an earlier part of the same disease spectrum. ALS is understood to have a presymptomatic phase in which the underlying disease activity in case of known mutations may be detected by genetic testing and biomarkers prior to overt clinical signs or symptoms.

Figure 34: Design of Study 303 (ATLAS). Study treatment (tofersen or placebo) is administered via intrathecal injection. The dosing regimen consists of three loading doses administered at 14-day intervals followed by maintenance doses once every 28 days thereafter.



[a] Measured using Siemens Healthineers NfL Assay, [b] Assuming other eligibility criteria are met, [c] Follow-up in Part A will end once 28 participants have been enrolled in Part B, [d] Part D was originally designed with a placebo-control but was transitioned to open-label following the sponsor's review of the results of the Phase 3 VALOR study. ALS amyotrophic lateral sclerosis, NfL neurofilament light chain
Source: Benatar et al (2022) "Design of a Randomized, Placebo-Controlled, Phase 3 Trial of Tofersen Initiated in Clinically Presymptomatic SOD1 Variant Carriers: the ATLAS Study".

The Study 303 primary analysis is expected to be in 2028.

No efficacy data from Study 233AS303 have been included in this application because the study remains blinded. Blinded safety data as of the 15 July 2022 safety data cut show no new safety signal. Data from Study 233AS303 will help inform the optimal timing of tofersen initiation.

Registry Collaboration

The collection and analysis of real-world data will play a vital role in expanding our knowledge of the natural history of SOD1-ALS and the effects of tofersen. The applicant is collaborating with 2 large, ongoing disease registry consortia/networks, the ALS/motor neuron disease NHC (primarily US-based) and the TRICALS Network (European sites), to fund prospective data collection efforts, including the collection of information related to the safety and effectiveness of tofersen.

- ALS/MND NHC Study is an established multidisciplinary clinic-based registry that prospectively and longitudinally captures important clinical information about the disease process from people living with ALS. The consortium consists of academic medical centres in the US (9 sites) and Europe (1 site in Italy).
- The applicant is collaborating with TRICALS investigators to develop the Precision ALS program to conduct a multinational, multi-centric, observational population-based study that will examine ALS natural history and outcomes, including those associated with existing or new disease-modifying treatments, prospectively in a real-world setting. As of September 2022, the applicant has an active 3-year contract to fund prospective data collection in Precision ALS.

In order to determine whether Precision ALS and/or the ALS/MND Natural History Consortium would be fit-for-purpose for a registry-based study to be conducted as a commitment to the European Medicines Agency (EMA), the applicant, through consultants, has conducted a feasibility assessment and submitted the relevant report. In that report it is stated that Precision ALS is a research initiative, recently funded through public-private partnership, for the development of a patient data platform. It is also stated that TRICALS will leverage the Precision ALS and the TRICALS centers to create a research platform, consisting of a longitudinal data collection infrastructure based on electronic medical records as well as primary data collection for some variables. It was concluded that based on the infrastructure and the type of data collected (with the possibility to implement additional data collection), it appears that a PASS on the safety and effectiveness of tofersen would be feasible within either registry. The number of countries that will be effectively included in the PASS will depend on the schedule of tofersen launch in Europe since it may not be launched at the same time in all countries covered by TRICALS.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The applicant has completed study 233AS101, consisting of phase 1/2 parts A and B and phase 3 Part C. Study 101 Part C is considered the single pivotal double-blind placebo controlled study. Upon request, several analyses have been provided and are included in the report. The OLE study 102 provided data up to 168 weeks. Data from two interim analyses (2nd interim analysis data cutoff 16 January 2022 after 52 weeks and 3rd interim analysis data cutoff 28 February 2023 after 104 weeks) are relevant for this discussion on efficacy. The present MAA was included in the Raw Data Pilot project which generated several additional analyses.

The design of the pivotal study 101 Part C as a randomised, double-blind, placebo-controlled study is appropriate. Given the inherent heterogeneity of SOD1-ALS and the fact that the decline on the ALSFRS-R in the placebo arm turned out to be three-fold lower than initially assumed (i.e., a decline by 8.1 points over the 6-month period rather than the anticipated 24.7 points; see also Section 2.6.5.2.1 of this AR), the 28-week duration of the study (selected based on the above-mentioned assumptions) was in view of the results generated apparently too short to overcome the disease heterogeneity in the population and show a convincing clinical treatment effect.

The patient population was focused on SOD1-ALS. However, there are over 200 variants in the SOD1 mutation making this small proportion of the general ALS population very heterogeneous. Forty-two unique SOD1 mutations centrally confirmed as pathogenic or likely pathogenic were enrolled. The most commonly identified SOD1 mutation types were p.Ile114Thr (n= 20/108; 18.5%), p.Ala5Val (n = 17/108; 15.7%), p.Gly94Cys (n = 6/108; 5.6%), and p.His47Arg (n = 5/108; 4.6%).

The ALSFRS-R scale was chosen as primary endpoint and is considered appropriate. The selected 28-week duration for the primary analysis is considered too short to show a clinical response for the reasons discussed in the previous paragraphs.

Protocol deviations in study 101 Part C were not considered to have affected the conclusions of the study. A GCP inspection concluded that the majority of the data reported for the 233AS101 Part C clinical trial are acceptable and the findings of the inspection do not have a substantial impact on the data of the clinical trial inspected.

The SAP versions describe in detail the planned statistical methods for data collected in the studies and subsequent *post hoc* analyses.

The JRT of survival and function difference after 28 weeks in the mITT population was the pre-specified primary analysis. The JRT is an established analysis for ALS and was an appropriate choice for primary analysis but failed to show a statistically significant difference between tofersen and placebo.

As summary statistics for the treatment effect on ALSFRS-R changes, the difference between treatment groups in LS means of Day 197 was provided based on ANCOVA+MI. Additional sensitivity analyses and *post hoc* analyses including alternative covariates than in the pre-specified ANCOVA model and imputation model were conducted, whereby the applicant particularly emphasizes the relevance of the analyses adjusting for baseline NfL due to an observed imbalance and the claimed importance of baseline plasma NfL as a prognostic factor. However, as stated in the EMA 'Guideline on adjustment for baseline covariates in clinical trials', a baseline imbalance in a covariate should not be considered as an appropriate reason to include this baseline measure as a covariate in the primary analysis. Even if it is acknowledged that NfL may be a prognostic factor, control of the type 1 error is ensured only for the pre-specified primary analysis, which should also be the starting point for considerations on the size of the effect.

With respect to the untransformed baseline NfL values, which were included in the applicant's ANCOVAs as a covariate, the applicant's response provides some reassurance on the robustness of the statistical results in relation to the scale used for baseline NfL adjustment. Log-transformation of NfL had no major impact on the time to death or PV analysis.

Missing data were replaced under a MAR assumption, which can be considered to leading to an analysis aligned to targeting the hypothetical effect 'if all patients had completed treatment until the analysis time point and survived'. From a regulatory point of view, targeting the treatment effect irrespectively of treatment discontinuation is of primary relevance. For targeting this effect, assuming loss of potential benefit from treatment appears to be plausible such that replacing missing data after treatment discontinuation using reference-based imputation appears appropriate. The appropriate strategy how to account for death is challenging but it could be considered as complete loss of function such that imputation of 0 may be meaningful. These alternative missing data strategies were explored within the raw data pilot and reproduced by the applicant.

Participants with SOD1-ALS who completed Part A, B, or C of Study 233AS101 were allowed to continue to the ongoing multicentre, open-label, extension study to further assess efficacy and safety in the long-term Study 102 LTE. Of the 159 eligible participants who completed Study 101, a total of 139 participants enrolled from Study 101 Parts A, B, and C: 44 participants enrolled from Study 101 Parts A and B and 95 participants enrolled from Part C.

The same functional scale and important endpoints as the ones used in study 101 Part C were used for evaluating the potentially long-term effect of tofersen in study 102 LTE.

A study in pre-symptomatic ALS patients is on-going. Study 233AS303 (ATLAS) is a Phase 3, randomized, double-blind, placebo-controlled, 4-part study designed to evaluate tofersen in presymptomatic carriers of a SOD1 mutation. No efficacy data from this study was submitted as part of the current application.

The applicant has also submitted a continuous evidence generation plan. (see below).

Efficacy data and additional analyses

The population enrolled in this study was representative of the broad SOD1-ALS population. It is noted that A4V/A5V (p.Ala5Val) mutation carriers are considered the most prevalent fast progressing mutation type enrolled which is consistently associated with a median survival (based on Kaplan Meier) of or below 1.2 years. There were 17 (16%) of them included in the study.

It appears that the tofersen group in general was in a worse condition than the placebo group. Mean values for ALSFRS-R baseline total score, % predicted SVC at baseline and plasma NfL at baseline

(pg/mL) were slightly worse in the tofersen group with 36.9, 82.1% and 100.4 pg/ml respectively, compared to the placebo group with values of 37.3, 85.13% and 89.7 pg/ml, respectively.

Study 101 Part C baseline plasma NfL levels in the ITT population were approximately 15% to 25% higher in the early-start tofersen group than in the placebo/delayed-start group at baseline. These higher plasma NfL levels in the early start group were not in favour of tofersen treatment.

In the pivotal study 101 Part C, no statistically significant difference in ALSFRS-R change from baseline to Week 28 was observed in the primary analysis mITT population (difference: 1.2 points favoring tofersen; 95% CI: –3.2 to 5.5; p = 0.97 via JRT + MI analysis). Hence, study 101 Part C has failed to provide confirmatory evidence of efficacy necessary to support a full marketing authorisation. Consequently, all other analyses provided from study 101 Part C need to be considered exploratory. The applicant performed several analyses including a *post hoc* analysis of ALSFRS-R with baseline plasma NfL as a covariate and found a statistically non-significant difference of 2.1 (95% CI: -0.3 to 4.5; p = 0.0904 via JRT + MI analysis) between tofersen and placebo in the ALSFRS-R total score change from baseline to week 28 in the ITT population.

With a reference based imputation analysis of the results (via the Raw Data Pilot Project), a smaller treatment effect (as expressed in difference between tofersen and placebo in the change of ALSFRS-R total score from study 101 part C baseline to week 28) was observed (J2R, not adjusted for baseline NfL: -0.97; 95% CI (-4.3,2.4), J2R, adjusted for baseline NfL: -1.7, 95% CI (-4.8,1.4)).

With respect to the analyses using the Raw Data Pilot Project, from a regulatory point of view, targeting the treatment effect irrespectively of treatment discontinuation is of primary relevance. For targeting this effect, assuming loss of potential benefit from treatment after treatment discontinuation appears to be more plausible than a MAR assumption such that replacing missing data after treatment discontinuation using reference-based imputation as in the analyses above appears appropriate. If the treatment were disease-modifying, it would appear plausible that some effect from active treatment is retained at least for some time after treatment discontinuation, which is more in alignment with CR or CIR. However, as the size of the possible retention is unclear, J2R providing a lower bound is also of relevance. As death could be considered as complete loss of function, 0 could be considered the true ALSFRS-R value after death. A smaller effect is observed when reference-based imputation analysis is implemented.

The applicant repeated the analysis. Minor differences between the analyses with the Raw Data Pilot project and the analyses by the applicant were observed but the differences are not critical and not considered to have a major impact for the conclusions of the clinical outcome.

In a prespecified analysis based on the baseline median NfL value of 75.6 pg/ml, numerically larger differences were observed between tofersen and placebo over 28 weeks in patients with baseline NfL values above the median [mean difference (95% CI 3.9, (-1.0;8.9)] compared to patients with baseline NfL values below median [0.6, (-1.3,4.2)].

Analyses of the secondary endpoints percent predicted SVC and HDD megascore showed small and not statistically significant differences between tofersen treatment and placebo at week 28 i.e. 8.5%, JR+MI p=0.0689 and 0.10 ANCOVA+MI p=0.1547, respectively. There was, however, a consistent numerical trend favouring tofersen.

With respect to survival, the percentage of patients in the mITT population (and consequently in the ITT population) the percentage for an event of death or PV was similar in the placebo (9.5%) and tofersen (10.3%) groups. However, the numbers from Study 101 Part C are too low to be considered meaningful (see also below).

The results of the quality of life PROs were not statistically significant but also showed a trend in favour of tofersen.

In conclusion, although there was a trend in favour of tofersen treatment, efficacy could not be demonstrated in a confirmatory way in the pivotal phase 3 study 101 Part C at week 28. The heterogeneity of the SOD1 population, the selection of suboptimal prognostic variables for enrichment purposes (e.g. pre-randomisation ALSFRS-R and type of mutations instead of baseline plasma NfL levels), and the short duration of the study are considered factors which contributed to no statistically significant difference being found on the primary endpoint in the pivotal trial.

In the study 102 LTE report, 2nd interim analysis (with data cutoff 16 January 2022 up to week 52), the applicant has summarised accurately the situation of the pivotal phase 3 study 101 Part C.

In Study 233AS101 Part C (Phase 3), administration of tofersen 100 mg for up to 28 weeks did not result in a statistically significant slowing of decline across measures of clinical function (ALSFRS-R), respiratory function (SVC), and muscle strength (HHD megascore) compared with placebo over 28 weeks. However, consistent trends favoring tofersen over placebo and suggesting a slowing of disease progression were observed in primary, secondary, and exploratory measures. Consistent with its intended mechanism of action, tofersen administration resulted in sustained reductions in total CSF SOD1 protein. Reductions in plasma NfL suggest tofersen is reducing axonal injury and motor neuron loss.

A mechanistic basis for the potential treatment effect of tofersen exists. There is a strong biological plausibility, that the lowering of toxic SOD1 exerts disease-modifying effects of tofersen, which can be pharmacodynamically measured by an appropriate neurodegeneration biomarker, i.e. NfL.

Exploratory analyses were prespecified in alternative disease progression subgroups defined according to baseline plasma NfL levels, whereby those with a baseline plasma NfL level greater than the median (75.6 pg/ml) comprised the faster-progressing subgroup (FPS [NfL-based]), and those with a baseline plasma NfL level less than the median (<75.6 pg/ml), the slower-progressing subgroup (SPS [NfL-based]). The applicant, recognizing that adjustment for baseline NfL as a continuous covariate could provide even greater precision to the estimated treatment difference than categorical subgrouping based on the median, prespecified also sensitivity analyses incorporating baseline plasma NfL as a covariate in the ANCOVA model for the disease progression subgroups defined by mutation and ALSFRS-R slope (FPS [mutation/slope] and SPS [mutation/slope]). These NfL-based analyses were planned and prespecified in the SAP [Study 101 Part C SAP Version 2.0, 14 August 2021] prior to analysis of data from Study 101 Part C. After analysis of data from Study 101 Part C, the integrated efficacy analysis plan for Studies 101 and 102 was amended, prior to analysis for the 16 January 2022 data cutoff, to include covariate adjustment for baseline levels of NfL in the analyses of the full ITT population (SAP Version 3.0, 02 February 2022). This approach was also utilized for the analyses of the most recent data cut (28 February 2023 data cut; 18 May 2023 database lock), which was conducted in accordance with the SAP Version 4.0, 17 May 2023.

The pooled analysis at week 52 (2nd interim analysis data cutoff 16 January 2022, ITT population)**,** consisting of the double-blind data from study Part C and the open-label data from study 102 LTE, showed a nominally statistically significant difference between the early and the placebo/delayed start tofersen treatment group of 3.5 (ANCOVA+MI $p_{nominal}$=0.0272) in ALSFRS-R total score when adjusting for baseline NfL. Additional analyses adjusting for alternative covariates and all subgroups show positive trends in favour of tofersen as well. The most important secondary endpoints 'percent predicted SVC' and 'HDD megascore' were also nominally statistically significant albeit the differences between treatment groups were small, i.e., 9.2% (ANCOVA+MI $p_{nominal}$=0.0159) and 0.028 (ANCOVA+MI $p_{nominal}$=0.0186), respectively. Two quality of life outcomes (change from baseline in ALSAQ-5 and change from baseline in ED-5D-5L utility score) were nominally statistically significant and two (change from baseline on FSS and on EQ-5D-VAS) showed trends in favour of tofersen.

The applicant has performed, upon request, an additional interim analysis for efficacy using as data cut 28 February 2023 (3rd interim analysis).

Ninety-five out of 108 participants (88%) randomized in Study 101 Part C went on to receive tofersen 100 mg in Study 102. In the early-start group (tofersen/tofersen), from baseline of study 101 Part C, 19 participants discontinued study due to death (n=10, 14%) or disease progression (n=7, 10%), while in the delayed-start group, 16 participants discontinued study due to death (n=7, 19%) or disease progression (n=5, 14%). Rates of study discontinuation due to death or disease progression are numerically more in favour of the early-start group (24%) when compared to the delayed-start group (33%) at Week 104. At the time of the 28 February 2023 data cutoff (3rd interim analysis), 16 participants (44.4%) in the placebo/delayed-start group and 44 participants (61.1%) in the early-start group remained ongoing in OLE Study 102.

Sustained reductions were observed in CSF SOD1 with 39% in the placebo/delayed-start group and 37% in the early-start group at week 76 and 19% in the placebo/delayed-start group and 27% in the early-start group at week 104. In the case of plasma NfL, robust reductions in the placebo/delayed-start group, in plasma NfL of 43% at Week 76 and 60% at Week 104 were observed. For the early-start group, reductions in plasma NfL were sustained, i.e. 55% at Week 76 and 66% at Week 104. NfL cannot be considered as a validated surrogate endpoint for predicting efficacy. However, consistent reductions in CSF SOD1 and plasma NfL, supported by nonclinical data are supporting the biological plausibility with target engagement and a strong mechanistic rationale for the effect of tofersen treatment.

The data suggest that these reductions in SOD1 protein and consequently NfL result in a delayed effect on function as measured by ALSFRS-R and other clinical outcome measures, which is considered biologically plausible. The applicant states "*highlighting the time needed for a slowing of neurodegeneration to translate to detectable slowing of clinical decline*". A delayed clinical effect is also supported by nonclinical findings.

Long-term data from studies 101 and 102 up to Week 104 indicate numerical differences in favour of early-start tofersen treatment on different assessments (e.g. ALSFRS-R and SVC) across several analyses of populations and covariates. With respect to the most important clinical endpoint on function, participants in Study 101 Part C and Study 102 lost 9.5 and 13.2 points (adjusted mean on ANCOVA+MI) over 24 months in the tofersen early-start and placebo/delayed-start tofersen group, respectively. It is estimated that people living with ALS decline by an average of ~1 point per month. The adjusted mean difference between groups was 3.7 (nominal ANCOVA+MI p=0.1004) and remained similar compared to week 52. The effects on respiratory strength seen at earlier timepoints were sustained at 104 weeks, with a 9.7 percent-predicted difference in SVC in favor of early-start tofersen. Tofersen treatment continued to favor the early-start group with a 0.19-point difference in HHD megascore.

The analyses at week 104 using the new data cut-off (28 February 2023) show some attenuation of the differences between treatment groups (i.e. between early-start and delayed-start of tofersen) that were previously observed at week 52 but a clear difference still exists further supporting a greater benefit with early treatment start. The data provided by the applicant suggest stabilisation or even some improvement in participants remaining in the study regarding clinical function, respiratory function, muscle strength and quality of life assessments (ALSFRS-R, SVC, HHD, ALSAQ-5, EQ-5D-5L) over 100/104 weeks, which would not be expected from the natural course of the disease. As highlighted by the applicant, there are some relevant limitations associated with comparisons with the natural history data (e.g. inter- and intra-mutation variability), and therefore, such comparisons should be interpreted with caution.

However, the differences observed between early start and placebo/delayed start tofersen treatment group at week 52 remain similar until week 104. This observed stabilisation would be supportive of a true disease modifying effect of tofersen.

Performing a responder analysis without logistic regression at week 100 (for patient-reported outcomes (PRO)) or week 104 (clinical measures), in the early-start tofersen group, 19.5% of participants experienced improvement on the ALSFRS-R, 21.4% improvement on SVC, and 25.8% improvement on HHD. In the case of placebo/delayed start tofersen group these improvements were 11.6% for ALSFR-R, 10.5% for SVC and 10.1% for HHD. A logistic regression model was used in the original analysis to allow the adjustment of baseline NfL, due to its prognostic value. Both analyses with or without logistic regression indicate a slowing of decline and even an improvement in function, strength, and QoL for the early and placebo/delayed start tofersen treatment group with a clear difference between groups and are rather unexpected for the natural course of the disease. Early initiation of tofersen treatment appears to be more beneficial compared to a delayed start. Some response is also seen on the placebo/delayed start tofersen treatment.

The cumulative distribution function analyses do not contradict the observed trends in favour of tofersen. Participants in the placebo/delayed-start group tend to have a higher rate of worsening in ALSFRS-R at Week 52. As an example, for a cutoff of -12, the rates of worsening in ALSFRS-R are approximately 32% and 44% for the early-start tofersen group and the placebo/delayed-start tofersen group, respectively.

Although the decline in ALS is not always linear, it is estimated that people living with ALS decline by an average of ~1 point per month, which can reflect the difference between being able to walk with assistance and being non-ambulatory.

Indications for stabilisation of the disease or even improvement have been observed in the case of rapidly progressing p.Ala5Val (A5V) SOD1 mutation carriers, which is also rather inconsistent with the usual progressive decline in these patients, who have a median survival of ~1 to 1.2 years.

Participants from Study 233AS101 Part A and B who ultimately received tofersen 100 mg in Study 233AS102 appear to have achieved stabilisation in clinical function for over 3.5 years since the start of the study with a median follow-up time of 4.7 years (range: 3.1 to 5.8 years). These participants experienced a mean change from Study 102 baseline to Week 168 (approximately 39 months or 3.2 years) of -2.3 points on ALSFRS-R total score, -2.4 on percent-predicted SVC, and -0.1 on HHD megascore. As discussed by the applicant, Part A/B participants (ABL) should be interpreted with caution due to the significant washout periods, variable dosing history, and having SOD1 mutation variants known to belong to the slower/intermediately progressing nature of the disease. However, even in slower-progressing SOD1-ALS patients, the disease is associated with progressive declines in strength and function.

There were also several analyses for event-free survival. The HR for the time to death, PV, or withdrawal due to disease progression was 0.50 and the HR for time to death or PV was 0.47. The applicant claims that the longer-term data show that the risk of death or PV was reduced. However, although numerical trends in favour of the early start tofersen group were observed, no conclusions can be currently drawn on an effect of tofersen on time to event-free survival. The reason is that robustness is highly questionable due to small event numbers and immature data and the strong assumptions made for the analysis.

Under the Raw Data Pilot project, a new time to event analysis was conducted with event defined as change from baseline in ALSFRS-R total score greater than 10 points (< -10), 21 days of PV assistance or death at any time point up to Week 124 (~17 months). Stable patients were those who did not present an event described above i.e. death, PV or deterioration of ALSFRS-R greater than 10 points (change of ALSFRS-R total score <-10). The HR for a "survival" event was 0.70 and upon adjusting for log(baselineNfL) HR was 0.46. Looking at the "distribution" of variants across groups it can be agreed that the survival benefit in the early-start participants, relative to the delayed-start participants, is unlikely to be attributed to enrollment of individuals with more slowly progressing disease.

Looking at the totality of data submitted, the following can be noted. SOD1-ALS is an ultra-rare disease (with approximately 1000 prevalent cases in EU) with a high unmet medical need. The placebo-controlled study failed to provide confirmatory evidence of efficacy of tofersen, as the pre-specified primary analysis did not reach statistical significance at week 28, in the change from baseline on the ALSFRS-R between tofersen and placebo regardless of the analyses conducted. However, all endpoints showed a trend in favour of tofersen. Consistently, these trends in favour of tofersen were observed at week 52 and at week 104, irrespective of the type of analysis performed.

It can be expected that reduction of the causative toxic mutant protein SOD1 in SOD1- ALS should lead to a reduction in neuronal axonal injury and neurodegeneration, which would subsequently stabilize or delay the progression of the disease. Treatment with tofersen, an ASO that specifically targets the production of SOD1, led to a relevant and sustained lowering of both SOD1 and NfL in plasma and CSF. Neurofilament has not been established as a surrogate endpoint for efficacy (or a worldwide acceptable reasonably likely surrogate endpoint) but is increasingly recognised as an important biomarker in neurodegenerative diseases, including ALS and is being used in most of the clinical trials in ALS. A number of references are available supporting the relevance of reductions in neurofilaments after active treatment (Thompson et al 2022, Brousse et al 2023, Jiang et al 2022, Mead et al 2023, McCluskey et al 2023 and Sanchez-Tejerina et al 2023). Researchers are advocating the use of neurofilament as being prognostic for disease progression across ALS populations. In another trial conducted with interleukin-2, FDA recommended that the prespecified analyses is adjusted for baseline neurofilament levels when analysing disease progression and survival. Recently, the EXPERTS-ALS program in the UK announced that NfL levels will be utilized to help screen potential drugs in ALS patients and select the best candidates to advance to larger Phase 3 trials [University of Sheffield 2023]. On the specific issue, the SAG-N experts considered by consensus that the level of NfL is a biomarker of axonal injury in ALS. The SAG-N experts also specified that NFL is not an ALS specific biomarker.

Although, the pivotal study 101C failed its primary objective at week 28, which is unsurprising considering the short placebo-controlled phase of only 6 months, favourable trends in the clinical endpoints were observed and were consistent over 104 weeks. Various analyses of survival and "survival-related" (or death-equivalent) events also suggest a benefit but cannot be considered robust, mainly due to the low number of events. However, the long-term data illustrate that tofersen-treated participants are exceeding the expected survival time as derived from natural history data, with the majority of participants being alive and ongoing in the study. Based on the results from the PROs, the apparently increased survival is not at the expense of reduced quality of life and using machinery to just keep the patients alive.

Data suggest that clinical effects are delayed compared to effects on NfL, which is considered biologically plausible. Examples of delayed effects of patient-customized treatments with ASOs in neurodegenerative disorders have become known for milasen (Kim et al. NEJM 2019) and jacifusen (for the patient Anna Kaempfer [preliminary presentation in https://www.nature.com/articles/s41591-021-01615-z]). The latter is an ASO directed against mutations in the FUS gene, which is another rare cause of genetic ALS (1% of ALS). The case was presented at the ALS workshop in Bonn 18 August 2023 (https://als-charite.de/fus-als-medikament-jacifusen-experten-und-angehoerige-im-podcast-der-n-lorem-foundation/). The specific treatment in this patient showed a delayed clinical response in a 16 year old girl with FUS ALS recovering from full mechanical ventilation to being able to walk and climb stairs again. A phase 3 trial (NCT04768972) is currently ongoing.

These cases support the postulation that ASOs can reduce toxic protein aggregates and consequently slow or even halt the neurodegenerative process, provided that they are administered for a sufficiently long period of time. It should be noted that the biomarker and clinical findings are supported by data in relevant animal models.

During the procedure, potential correlations between changes in NfL from baseline to week 16 (when the full effect on NfL had been achieved) and changes from baseline to week 28 (placebo-controlled phase) in ALSFRS-R were explored in the Raw Data Pilot Project. While only weak correlations were observed in the Raw Data Pilot Project, the correlations were stronger in the applicant's model with causal inference component. The applicant argued that adjustment for baseline NfL is necessary in the correlation analyses to take into account the variable natural disease progression across participants. The justification is considered plausible; however, uncertainty remains regarding the level of association between reductions in NfL and slowing of disease progression, and these results are considered to remain hypothesis-generating only. Correlation coefficients adjusted for baseline NfL showed modest inverse correlations between change in NfL levels at week 16 (when the full effect on NfL had been achieved) and change in ALSFRS-R functional score at week 28 (end of placebo-controlled study), which became stronger at week 52, which is according to the expectation when there is a delayed clinical effect following the reduction in neuronal loss.

Neither the size of the (partial) correlation coefficients nor their statistical significance provide added value with regard to the evaluation of the clinical relevance of the effects. However, the partial correlations are in line with the hypothesis of a relationship between changes in NfL and clinical outcomes such that the previously apparent inconsistency between the correlation levels observed in the Raw Data Pilot Project and the statistical results of the applicant's model with causal inference component is resolved.

The majority of the SAG-N experts considered that NFL levels can be used for predicting a clinical effect in patients with ALS, at least on a cohort level (for details see below section on Additional expert consultation).

Additional long-term clinical data were submitted during the procedure. An extended duration of follow-up from baseline to 3.4 years showed that the majority of patients remain alive on-study, since the number of death-equivalent events were limited. The range of follow-up from 2.2 to 3.9 years certainly exceeds the 2.3 years median survival for SOD1-ALS characterized in the literature. This finding further supports the efficacy of tofersen.

A case of an EAP participant with a relatively quickly progressing SOD1 mutation was also presented. This individual experienced a maintenance of a stable score on ALSFRS-R over 1 year of treatment and improvements on the timed up and go and 10-meter walk test. Although this is an isolated case within the EAP, it suggests a benefit of tofersen treatment for the person concerned, especially as this person was characterised as progressing relatively quickly.

SOD1-ALS, accounting for only approximately 2% of all ALS cases, is a serious, progressive and invariably fatal disease. In the CHMP view, the totality of data illustrates the potential of tofersen to slow disease progression, improve strength, function and QoL and finally prolong survival in these patients, that clearly exceed the expected natural history of the disease. The targeted MoA and supportive non-clinical results further strengthen the plausibility and likelihood of tofersen being an effective treatment for SOD1-ALS. However, as the CHMP considered that the data included in the application is not comprehensive as required for a full marketing authorisation, the CHMP has thoroughly discussed the applicable requirements for either a conditional marketing authorisation (CMA) or a Marketing Authorisation under Exceptional Circumstances (MAUEC) and whether the specific obligations (SOBs) proposed by the applicant can provide comprehensive data to confirm the positive benefit-risk balance of the medicinal product (see Additional efficacy data needed in the context of a MA under exceptional circumstances as well as Section 3.7.3 Additional considerations on the benefit-risk balance).

Generation of additional/comprehensive data

See below under Additional efficacy data needed in the context of a MA under exceptional circumstances.

Additional expert consultation

A SAG-Neurology meeting enriched with ALS experts, was convened by the CHMP.

1. Is NfL considered to be a biomarker reflecting neuronal injury in ALS?

The SAG-N experts considered by consensus that the level of NfL is a biomarker of axonal injury in ALS. The SAG-N experts specified that NFL is not an ALS specific biomarker.

2. Do you believe NfL can be used for predicting a clinically relevant effect in patients with ALS?

The majority of the SAG-N experts considered that NFL levels can be used for predicting a clinical effect in patients with ALS. In the view of some SAG-N experts, it is uncertain whether NFL can be used for predicting a clinically relevant effect in ALS because there is no clear definition on what constitutes a clinically relevant effect in ALS (e.g., a threshold of change in a clinical scale) besides a change in survival which is considered as clinically relevant by all SAG-N experts.

Further, all restricted SAG-N experts considered that NfL levels can be used for predicting a clinically relevant effect in ALS on a cohort level. The distinction between cohort level and individual level was made in the view of the wide range of NfL levels. Among the SAG-N restricted experts, divergent views were expressed about the value at individual level for which only a single restricted SAG-N expert was clearly in favour of its use. Further, some restricted SAG-N experts recognized that timelines -time since the NFL change to the occurrence of the clinical effect - are unclear. Finally, one SAG-N restricted expert indicated that the type of mutation could be relevant but even this expert agreed on the predictive value on a general basis.

3. Is the available evidence sufficient to conclude that the observed reduction in plasma NfL in Tofersen-treated patients can translate into a clinical benefit in patients with SOD1-ALS?

Overall, the majority of the SAG-N experts agreed that there is evidence -although not a strong one- supporting that the observed reduction in plasma NfL in tofersen-treated patients can translate into a clinical benefit in patients with SOD1-ALS. A minority of the SAG-N experts expressed that there is no evidence. The results from the VALOR RCT do not allow for concluding that a reduction in plasma NfL can translate into a clinical benefit. However, the biological plausibility, the consistency of all clinical outcomes with point-estimates in favour of the treatment, and results from the open label extension (OLE) phase are highlighted as reasons supporting the position that reduction in plasma NfL can translate into a clinical benefit. However, the SAG-N experts also noted that there is some uncertainty in the prediction mainly due to the rarity of the events, the high heterogeneity of the population, and the short duration of the trial.

4. Does the available clinical and non-clinical data support a treatment effect of tofersen in SOD1-ALS patients?

The SAG-N experts agreed by consensus that the evidence from the double-blinded phase in VALOR study is not strong. A short study duration was highlighted as the most relevant limitation likely contributing to the absence of statistically significant effect on the primary endpoint. However, the SAG-N experts highlighted not only the results on the changes in NFL and SOD1 but also the positive trends in secondary clinical outcomes including respiratory function -a relevant aspect in ALS-in favour of the tofersen arm in the VALOR trial and the results in the OLE phase strongly suggesting treatment effects. None of these effects were statistically significant in the mITT population in the double-blinded randomized phase. All experts acknowledged that open label extension studies are as a general principle prone to bias.

Further, the majority of the experienced restricted SAG-N experts highlighted their clinical experience with tofersen reporting stabilisation of the clinical course in individual patients in clinical practice, an outcome that these experts never saw before in their vast clinical practice over the last years.

The patient representative acknowledged the limitations of the study results. However, the patient representative highlighted the undisputed unmet medical need for ALS and the positive results not only in the trial but also in the clinical practice as shared by the restricted SAG-N experts.

5. Are the design and endpoints of the ATLAS study in presymptomatic SOD1 mutation carriers considered suitable to provide confirmatory evidence for efficacy of tofersen in patients with symptomatic SOD1-ALS?

Overall, the SAG-N experts are not convinced that ATLAS study in presymptomatic SOD1 mutation carriers could provide confirmatory evidence for efficacy of tofersen in patients with symptomatic SOD1-ALS. The uncertainty is based on several limitations. First, it was considered that ATLAS will recruit presymptomatic SOD1 mutation carriers while VALOR study has been conducted in SOD1-ALS patients. However, one SAG-N restricted expert clarified that presymptomatic SOD1 mutation carriers will be randomized once they become (early) symptomatic. Second, the study population for the randomized clinical trial in ATLAS (n=28) is considered too small in the view of the heterogeneous SOD1-ALS population. Third, it was noted that not all SOD1 mutations are considered in the study (only mutations associated with rapid progression are included), and it is unclear whether results of ATLAS could be extrapolable to all SOD1 mutations. In this regard, one SAG-N restricted expert provided a rationale on some exclusions: mutations that are not thought to be pathogenic were excluded, mutations that have not ≈ 100% penetrance were excluded, and mutations associated with a very low progressing course (some genotypes associate life expectancy beyond 10 years) were also excluded.

The SAG-N discussed the possibility of a new well-designed RCT with sufficient statistical power, which would require a longer follow-up than the VALOR trial and a larger sample size that the ATLAS trial. Overall, the SAG-N experts considered that conducting such a new randomized controlled trial is not feasible given the current situation. Feasibility is unlikely, because many ALS-experts are convinced that tofersen is effective in SOD1-ALS and are currently prescribing it within a compassionate use program. Furthermore, the rarity of the condition was acknowledged, and it was highlighted by one of the restricted SAG-N experts that the possibility of recruiting more patients is further limited because not all mutations leading to SOD1-ALS could be considered eligible (e.g. mutations that are not pathogenic should not be included in his views).

At this stage, the majority of the SAG-N experts considered that is very unlikely that we will have comprehensive evidence in SOD1-ALS.

Additional efficacy data needed in the context of a MA under exceptional circumstances

The initial application was for a "full" marketing authorisation. The CHMP concluded the data are not comprehensive, even if they would finally be sufficient for approval.

The CHMP requested the applicant to discuss the plan for generation of additional post-authorisation data and whether such data will result in a comprehensive dossier.

The applicant has carefully considered alternative ways to generate additional data and whether these could be deemed comprehensive by the CHMP. The applicant provided justification in support of a MAUEC, including justification for the inability to provide comprehensive data under normal conditions of use. The SAG-N experts also remarked that: "*At this stage, the majority of the SAG-N experts considered that it is very unlikely that we will have comprehensive evidence in SOD1-ALS*". Based on the totality of the data to be generated through the proposed plan, the CHMP agreed on a MAUEC as being

the appropriate type of marketing authorisation because of the rarity of the disease and the improbability that comprehensive data for a full marketing authorisation can be generated.

The applicant has pointed out that additional placebo-controlled studies in symptomatic SOD1 ALS are not considered operationally feasible. This is agreed, given the ultra-rarity of the disease and especially if tofersen would be approved. In particular, as the condition is SOD1-ALS, only a small subset of all ALS patients could be considered eligible for a new trial. Therefore, the epidemiology of the condition *per se* challenges the feasibility of conducting a new trial. Second, it is also agreed that the approval and the subsequent commercialisation of tofersen could impact the ability to recruit patients in a placebo-controlled trial, because ALS is a fatal condition for which the existing therapeutic armamentarium is very limited. As pointed out by the SAG-N, many ALS-experts are convinced that tofersen is effective in SOD1-ALS and are currently prescribing it within a compassionate use program. Consequently, the CHMP agreed that conducting additional placebo-controlled studies in symptomatic SOD1-ALS is unlikely feasible.

In the frame of the discussion concerning the data that can be generated post-approval, the applicant proposed a continuous evidence generation plan based on the Study 303, ongoing Study 102 and an observational registry-based study.

Table 34 shows the agreed SOBs. Specific milestones have also been proposed by the applicant. Moreover, the applicant will submit yearly updates on any new information concerning the efficacy and safety of tofersen (fifth SOB). It is expected that the agreed SOBs will provide important additional data to further support the benefit-risk profile of tofersen and additional insight in SOD1-ALS.

Study 233AS303 which is an ongoing phase 3, randomized, double-blind, placebo-controlled, 4-part study designed to evaluate whether tofersen, when initiated in presymptomatic carriers of a SOD1 mutation with biomarker evidence (increased plasma NfL) of disease activity, can halt or delay the emergence of clinically manifest ALS and/or slow the decline in function once symptoms are observed compared to the initiation of tofersen at the time of, or after, the emergence of clinically manifest ALS (see section 2.6.5.7.2). The CHMP highlighted several uncertainties with this study. First, the CHMP noted that the time from NfL elevation to emergence of clinically manifested ALS (CMALS) could be longer in non-A5V carriers. Thus, the applicant has amended the primary analysis timepoint from 12 to 24 months, which impacts the timing of data availability (expected Q4 2028). Another uncertainty is the need to evaluate potentially confounding factors for neurofilament elevations, e.g. other concomitant diseases that lead to NfL elevations such as prion diseases, frontotemporal dementia, vascular dementia, multiple systems atrophy, progressive supranuclear palsy and others (Gordon 2020, Yuan and Nixon 2021). NfL elevations are a nonspecific indicator of neurodegeneration, and investigators will be required to assess for alternative (unrelated to ALS) identifiable causes for an elevation when a participant's NfL level meets the protocol-defined criteria. There is also the possibility that more frequent monitoring of NfL will lead to earlier detection of disease activity and therefore to an earlier diagnosis of symptomatic SOD1-ALS (compared to the pre-symptomatic familial ALS [Pre-fALS] study). According to the study design, subjects who become symptomatic before reaching the NfL threshold are assigned to part D where they will receive tofersen. Efficacy data from this uncontrolled open-label study arm D, although valuable, will not provide confirmatory evidence of efficacy. Additionally, Pre-fALS is a prospective natural history and biomarker study of people not yet affected with ALS but who are at genetic risk for developing ALS (NCT00317616). The investigators aim to recruit unaffected (healthy) people from familial ALS (fALS) pedigrees in whom a known genetic mutation associated with ALS has been identified (see more details above). Of note, different mutations carry different prognoses in ALS. The most frequently reported mutation associated with fALS in Europe is C9orf72 mutation and not mutations leading to SOD1-ALS. It is unclear if the assumption underlying the NfL trajectory model to inform the design of study 303 which are based on the Pre-fALS study is reliable. Furthermore, ATLAS is enrolling an enriched population of presymptomatic SOD1-ALS carriers (based on selected variants of SOD1 mutations thought

to have high or complete penetrance and are associated with rapid disease progression), which casts doubts on the extrapolability of the results towards the broad SOD1-ALS patient population with symptomatic disease, which reflects the indication to be authorised (as also highlighted by SAG-N experts). The presymptomatic population to be studied in study 303 (apart from the symptomatic patients in Parts C and D) is clearly different from the population in the indication. A demonstrated tofersen treatment benefit in this presymptomatic population would certainly be supportive of the approved indication, but this study is not expected to generate a comprehensive dossier as it would not generate confirmatory evidence of efficacy in the authorised indication. Finally, the sample size of 28 patients in the placebo-controlled part B is small such that a treatment effect can be shown only if it is large. This has been highlighted as the most relevant limitation by the SAG-N experts. The applicant discussed whether an enlargement of the sample size of the placebo-controlled part B of study 303 is possible in order to increase the likelihood of the study to provide confirmatory evidence of treatment effect under trial conditions. Various changes have been explored by the applicant, upon request from CHMP. According to the applicant, changes such as expanding eligibility to slower progressing variant carriers, increasing the study sample size, etc. are not expected to meaningfully improve the likelihood that data from Study 303 would be considered comprehensive, but would rather have a substantial impact on feasibility and study timelines.

The applicant's view is that the ability of Study 303 to provide comprehensive confirmatory data post-authorisation remains uncertain. This view is also shared by CHMP and the SAG-N experts. In addition, the feasibility of completing the ATLAS study as planned once tofersen is approved has also been addressed. The applicant provided the latest update of the status of the on-going study 233AS303. Study 303 is well in progress with 68% of the planned presymptomatic at-risk carriers of SOD1 mutation already enrolled. Based on the current study design and enrollment rate in the interventional study period (Part B), data from Study 303 are anticipated in 2028. There is no plan for an interim analysis of Study 303. The applicant is committed to completing study 303 and is confident for several reasons that approval in Europe will not impact the feasibility of completing the study.

The CHMP concluded that the data expected to be generated by this study cannot provide comprehensive evidence due to the limitations and uncertainties described above. However, the CHMP is of the opinion that this study could provide relevant information on whether treatment with tofersen should already be initiated in asymptomatic carriers of SOD1 mutations (part B). Therefore, the tofersen treatment in presymptomatic SOD1 mutation carriers could provide additional data supporting efficacy of tofersen. Further, participants in study 303 who become symptomatic before reaching the NfL thresholds for enrolling part B (meaning they become SOD1-ALS patients) will remain in the study part D receiving tofersen. Therefore, efficacy data from these patients is considered valuable. Upon approval of tofersen, study 303 will be a SOB. Last patient out is expected in Q4 2027, and the final report of the study is scheduled for Q4 2028 (please see Table with Specific Obligations).

With regards to the extension **Study 233AS102**, it is agreed that the study will provide meaningful data in further characterising the benefits and the risks, in particular with regards to long-term safety (primary aim). It is also agreed that the longer follow-up will allow to provide more information on survival. However, due to the limitations of the design (the study is not a placebo-controlled trial; all patients will be treated (open label)), this would not generate data that would allow to provide confirmatory evidence of the clinical benefits of the medicinal product. Therefore, the results of this study are not expected to lead to a comprehensive confirmatory dataset.

With regards to **Study 233AS401**, collaborations with two large ongoing disease registries, including Precision-ALS in Europe (TRICALS), have already been initiated. From the applicant's experience, the successful management of high-quality and sustainable registry-based studies take time and can be resource intensive for the institutions. Given these known challenges, the applicant proposes to focus

initial efforts on the current collaborations and successful implementation of the Precision-ALS data platform before expanding further.

The applicant has been under discussions with NHC to fund prospective data collection with the ALS/MND NHC consortium, which consists of academic medical centres in the US and Europe. ALS-NHC is active and data reports and data cuts will be received every 6 months for the assessment of data quality and completeness. TRICALS is the world's largest network of specialty ALS centres which, through a common data model, collects and combines patient-level data from 47 centres in 15 European countries. Moreover, the applicant informed that registry contracts are in place with TRICALS and ALS-NHC. Approximately 2000 incident ALS cases (all ALS types) per year will be recruited to this bespoke Patient Data Platform, supported by the Precision ALS Program. Once established, the data collection will continue indefinitely as part of an ongoing TRICALS initiative and will be stored on a bespoke patient data platform designed by the Precision ALS Program. As this is a population-based study, existing prevalent cases will also be included, and patients will be recruited during routine clinic visits. The applicant have already provided information for their indefinite collaboration with the two registries in their continuous evidence generation plan document.

However, study 233AS401 is an observational registry-based study, and as such, the ability of this study to provide comprehensive data is limited compared to a well-designed and well-conducted placebo-controlled trial due to the lack of randomisation and blinding. The outcomes in treated patients may need to be compared with the outcomes in untreated patients. In essence, a comparison with a natural history cohort requires that natural history is characterized with high certainty which is not the case in this heterogeneous SOD-ALS population. Since the interpretation of the causal effects depends on a comparison with natural history which is not well characterised, the potential to interpret the observed effects as causal effects is questionable. Therefore, CHMP concluded it is unlikely that the data generated from this study will provide a comprehensive dataset.

Table 34: Applicant's proposed List of specific obligations (SOBs) in the context of MA under exceptional circumstances

Study Status	Summary of Objectives	Milestones	Due Date
233AS102 An Extension Study to Assess the Long-Term Safety, Tolerability, Pharmacokinetics, and Effect on Disease Progression of BIIB067 Administered to Previously Treated Adults with Amyotrophic Lateral Sclerosis Caused by Superoxide Dismutase 1 Mutation **Ongoing**	*Primary Objective:* • Evaluate the long-term safety and tolerability of BIIB067 in participants with SOD1-ALS. *Secondary Objectives:* • Evaluate the PK, PD, biomarker effects, and efficacy of BIIB067 administered to participants with ALS and confirmed SOD1 mutation.	Final report	30 September 2025
233AS303 A phase 3 randomized, placebo-controlled study in clinically presymptomatic adults with a confirmed *SOD1* mutation **Ongoing**	To investigate if initiation of tofersen in presymptomatic *SOD1*-ALS patients can delay or even prevent emergence of CMALS	Last Patient Out	31 December 2027 [a]
		Final report	31 December 2028

Study Status	Summary of Objectives	Milestones	Due Date
Integrated Analysis of Variant-Specific Survival **Planned**	Descriptive analyses of disease duration (survival) by *SOD1* variant-type in tofersen-treated (Studies 101/102; disease registries) vs. untreated patients (disease registries, natural history datasets/literature) Combined data from: • Study 101 (A, B, C) • Study 102 • Disease registries (ALS/MND-Natural History Consortium [NHC] and Precision-ALS) • Natural history datasets/literature	Initial analysis and output	30 June 2027 [b]
233AS401 An observational registry-based study to evaluate the long-term safety of tofersen in patients with *SOD1*-ALS **Planned**	Primary Objectives: • Describe demographic and clinical characteristics of *SOD1*-ALS participants at baseline and stratified by tofersen treatment status. • Estimate the incidence of serious adverse events (SAEs) among all participants and stratified by tofersen treatment status, including serious neurologic events previously reported in clinical trial participants (e.g., myelitis, radiculitis, aseptic meningitis, increased intracranial pressure, and/or papilledema) Secondary Objectives: • Disease progression measured by the ALSFRS-R as rate of change over time in total scores • Occurrence of relevant clinical outcomes, including permanent ventilation and feeding tube placement as well as changes in pulmonary function measured by FVC and SVC • Variant specific survival • Reports of new comorbid conditions, pregnancy and pregnancy outcome, and hospitalisations (including reason for hospitalisation) not reported as SAEs • Treatment discontinuation	Protocol submission	Within 6 months after the European Commission's decision on this application
		Interim reports	12 months after the Agency's approval of the final protocol and annually thereafter
		Continued evaluation	Annually (with annual reassessment)
Yearly updates on any new information concerning safety and efficacy of tofersen **Planned**	In order to ensure adequate monitoring of safety and efficacy of tofersen in the treatment of patients with SOD1-ALS, the MAH shall provide yearly updates on any new information concerning the safety and efficacy of tofersen.	Annual report	Annually (with annual reassessment)

a Dependent on enrollment timeline for Part B
b To be confirmed pending finalisation of registry collaboration agreements and consolidation of available natural history datasets

See also section 3.7.3 for details on the criteria of comprehensiveness.

2.6.7. Conclusions on the clinical efficacy

Tofersen, an ASO designed to bind to and degrade SOD1 mRNA, leads to reduction of the synthesis of toxic mutant SOD1 protein in patients with SOD1-ALS. The mechanism of action of tofersen is therefore targeted and well-defined.

Reducing toxic mutant SOD1 in SOD1-ALS may be expected to reduce neuronal injury and death. Neurofilaments are an integral component of neurons and an accepted biomarker of neurodegeneration.

Treatment with tofersen was shown to induce relevant and persistent reductions in SOD1 and subsequently in neurofilaments, suggesting target engagement and reduction in neuronal loss.

Despite its clear biological activity, tofersen did not significantly improve function in patients with SOD1-ALS in the 28-week single pivotal trial in comparison to placebo. This may be explained by a lag-time between effects on biomarkers and clinical effects, which appears biologically plausible and is supported by anecdotal evidence from two other ASOs used in patients with neurodegenerative diseases, including another form of ALS. Indeed, longer-term results showed consistent favourable effects on functional, strength, QoL and survival-related endpoints for early start vs. delayed start tofersen treatment, irrespective of the analyses performed and over long periods of time, at least up to week 104. In addition, the reported survival times in treated patients clearly exceed those expected from natural history data.

The targeted MoA and supportive non-clinical results further strengthen the plausibility and likelihood of tofersen being an effective treatment for SOD1-ALS.

Although, efficacy of tofersen in SOD1 ALS has not been proven in a confirmatory sense all results are consistent with a reasonably likely disease-modifying treatment effect.

Since the data are not considered comprehensive, and in view that the applicant has provided convincing arguments of inability to provide comprehensive data under normal conditions of use, it was therefore concluded that the recommendation of a MAUEC is appropriate.

The CHMP considers the following measures (SOB) necessary to address the missing efficacy data in the context of a MA under exceptional circumstances:

- 233AS102 An Extension Study to Assess the Long-Term Safety, Tolerability, Pharmacokinetics, and Effect on Disease Progression of BIIB067 Administered to Previously Treated Adults with Amyotrophic Lateral Sclerosis Caused by Superoxide Dismutase 1 Mutation.
- 233AS303 A phase 3 randomized, placebo-controlled study in clinically presymptomatic adults with a confirmed SOD1 mutation.
- Integrated Analysis of Variant-Specific Survival Descriptive analyses of disease duration (survival) by SOD1 variant-type in tofersen-treated (Studies 101/102; disease registries) vs. untreated patients (disease registries, natural history datasets/literature).
- 233AS401 An observational registry-based study to evaluate the long-term safety of tofersen in patients with SOD1-ALS.
- Submission of yearly updates on any new information concerning the safety and efficacy of tofersen

2.6.8. Clinical safety

Clinical safety data for this application are drawn from:

• 2 clinical studies conducted in participants with SOD1-ALS:

- Study 101 (completed; randomised, placebo-controlled in all three study parts, i.e. Part A [single ascending dose], Part B [multiple ascending dose], and Part C [constituting the pivotal study for this application]), and

- Study 102 (ongoing open-label extension for participants, who completed any part of study 101);

• a phase I study in healthy volunteers (233HV101), and

• an ongoing EAP.

A third clinical study (Study 303), evaluating tofersen vs. placebo in pre-symptomatic SOD1 mutation carriers is ongoing and still blinded; therefore, no detailed safety data are available for this study.

The cut-off date for safety and immunogenicity data originally submitted with the application is 15th July 2022, updated safety data for the ongoing study 102 and the EAP as of the 28th Feb 2023 have been provided with the responses to the D120 LoQ.

The primary focus of the safety evaluation is set on the comparison of safety in the active treatment group of study 101 Part C, receiving 100 mg tofersen each ('**Pool RC1**'), with the respective placebo group ('**Pool RC2**'), as well on the pooled safety data of the total of subjects receiving at least one dose of tofersen 100 mg during either part of study 101 or 102 (**Pool ABCL1**). Where relevant, reference is also made to the pooled population receiving at least one dose of tofersen (≤ 100 mg) during either part of study 101 or 102 (**Pool ABCL2**) and to all participants of study 101 Part C, who received tofersen 100 mg either during study 101 part C or during the extension study 102 (**Pool CL**).

2.6.8.1. Patient exposure

As of the 28 Feb 2023, 166 participants with ALS were exposed to any dose of tofersen for overall 408.82 participant-years (Pool ABCL2). Only four ALS subjects had no SOD1 mutations, all four were treated in the single-dose part A of study 101 only.

The median total exposure to any dose of tofersen was 131.7 weeks with a minimum exposure of 4 and a maximum exposure of 316 weeks (Pool ABCL2). A total of 147 participants from studies 101 and 102 with ALS were exposed to 100 mg of tofersen for 368.83 participant-years (Pool ABCL1). The median total duration of exposure to tofersen 100 mg for this pool was 148.4 weeks. In Pool ABCL1, the median number of doses received (via lumbar puncture procedures each) was 33, seven subjects received 56-60 and one subject received 61 doses.

An EAP is ongoing. As of 28 Feb 2023, patients from at least 13 countries have enrolled in the EAP. As dose administration is not tracked in the EAP, the number of tofersen shipments is used to approximate tofersen exposure. It is estimated, that overall, 287 SOD1-ALS patients were exposed to tofersen in the EAP. 67 EAP participants may have received tofersen for a year or longer up to approx. two years.

All safety results from the EAP were presented based on the data available in the global safety database. A total of 169 AEs (113 non-serious and 56 serious) were reported in 88 cases. The most frequently reported (≥ 4 events) AEs at the event level were post lumbar puncture syndrome (10), back pain (7), pneumonia aspiration (6), and procedural pain, headache, amyotrophic lateral sclerosis, COVID-19, CSF protein increased, diarrhoea, and respiratory failure (4 events each). Overall, 47 events in 34 cases reported at least 1 AE assessed as related or possibly related to tofersen by the health care professional (HCP). The most common (≥ 2 events) AEs related to tofersen were CSF cell count increased, intercepted product dispensing error, and post lumbar puncture syndrome (3 events each); and back pain, COVID-19, CSF lymphocyte count increased, CSF white blood cell count increased, disease progression, gait disturbance, meningitis aseptic, myalgia, and myelitis (2 events each). All AEs reported from the EAP pertaining to CSF parameters (n=15) were assessed as tofersen related and nonserious. The following AEs were also assessed as tofersen related: One serious event each of radiculopathy and papilloedema and 1 nonserious event of lumbar radiculopathy (radiculitis).

A total of 15 cases from the EAP reported a fatal outcome. These included a fatal event (PT) of death with minimal information reported and causality not assessed by the health care professional; the causality was therefore reported as related. None of the fatal events was assessed as related to tofersen by the HCP. The most common SOCs with fatal events (≥ 5 events) were Respiratory, thoracic, and mediastinal disorders, with PTs of respiratory failure (4 events), acute respiratory failure (2 events),

respiratory distress (1 event), bronchial secretion retention (1 event), and hypoventilation (1 event), followed by the system organ class (SOC) of Infections and infestations, with PTs of pneumonia aspiration (3 events), sepsis (1 event), and parotitis (1 event, temporally related to a myobloc injection).

A total of 56 SAEs were reported in 39 cases from the EAP. The most frequently (≥3) Serious AEs (SAEs) reported by PT were pneumonia aspiration (5), respiratory failure (4).

Eleven SAEs in 8 cases were assessed by the HCP as related or possibly related to tofersen. These concerned myelitis (2 cases); gait disturbance, speech disorder, and posture abnormal (1 case); meningitis aseptic (1 case); meningitis aseptic and papilloedema (1 case); post lumbar puncture syndrome (1 case); radiculopathy (1 case); and intervertebral discitis (1 case). Relevant information on related SAEs is depicted below:

• Myelitis (one Case) presented with symptoms of headache and tingling in the legs after the first and second doses and left-sided numbness and leg weakness 2 days after the third dose. MRI of the spine demonstrated multiple areas of demyelination, and CSF pleocytosis was noted. Tofersen was temporarily discontinued, and as of the 28 Feb 2023 data cut, it is unknown if tofersen has been resumed and if the event has resolved.

• Myelitis (one Case) became symptomatic 2 days after the 6th tofersen dose and was accompanied by CSF protein increase and cervical and thoracical contrast enhancement and oedema in the MRI. Tofersen was permanently discontinued, and the event resolved with sequelae of reduced strength endurance performance and improved but persistent weakness.

• Radiculopathy (one Case) including CSF pleocytosis and protein increase and contrast enhancement of the roots of the cuada and subpial medullary cone without sign of myelitis in the MRI was diagnosed in a patient who reported subacute worsening of their motor function. As of 28 February 2023, the outcome of the event of radiculopathy and action taken with tofersen as a result of the event remained unknown.

• The three SAEs of meningitis aseptic and/or papilloedema in two patients (two Cases) were not resolved as of the 28 Feb 2023, tofersen administration was maintained in both patients.

• Intervertebral discitis was reported in 1 case and was assessed by the HCP as possibly treatment related. The patient underwent MRI for an increase in chronic lumbago symptoms, which had been present for 10 years. MRI results demonstrated findings in the subchondral spongy bone at L4 to L5 and L5 to S1 levels, partially extending to the interbody discs. The event resolved completely without antibiotic therapy or other intervention. According to the narrative, tofersen had been temporarily stopped as a result of this SAE and was later resumed.

• Gait disturbance, speech disorder, and posture abnormal were reported in 1 case. The HCP considered these events to be related to tofersen because they occurred within 24 hours of injection and there was no evidence of inflammation or other alternative cause for the symptoms. The patient had a confounding medical history of ALS with an additional cerebellar ataxia component since April 2018.

• Hallucination visual and hypokalaemia were reported in 1 case. As causality assessment was not provided by the HCP, the events were reported as related in the original submission. Updated data (as of 28 Feb 2023) revealed that the patient experienced further of such episodes (without hypokalaemia) with subsequent doses. All episodes resolved within 24 hours, apparently decreased in severity with subsequent administrations, improved with anxiolytics and were rather considered suggestive of anxious manifestations and assessed as probably unrelated to tofersen by the HPC. MRI and electroencephalogram were without abnormal findings.

AEs leading to discontinuation of tofersen treatment from the EAP were reported in 14 cases (21 AEs). Adverse events by PT that led to study treatment discontinuation reported in ≥ 2 cases were pneumonia aspiration, amyotrophic lateral sclerosis, acute respiratory failure, and disease progression (2 cases

each). Of note, 1 serious event of myelitis and 1 nonserious event of lumbar radiculopathy led to discontinuation of tofersen. Action taken was unknown for 22 AEs and not applicable for 18 AEs.

AEs leading to interruption of tofersen from the EAP were reported in 9 cases and included influenza (1 case), intervertebral discitis (1 case), pneumonia aspiration (1 case), myelitis (1 case), femur fracture and fall (1 case), thrombocytopenia (1 case); intervertebral disc protrusion and gait disturbance (1 case); deep vein thrombosis (1 case); and pulmonary embolism (1 case). The event of thrombocytopenia was non-serious and assessed by the HPC as unrelated to tofersen but secondary to a helicobacter pylori infection.

2.6.8.2. Adverse events

An overview of adverse events reported during study 101 Part C, and Pool CL as well as the total tofersen population (Pools ABCL1, 2) is presented in Table 35.

Table 35: Overall summary of adverse events – safety population (cut-off: 28 Feb 2023)

	RC: Study 101 Part C (Part C subjects) placebo-controlled period		CL: Study 101 Part C and Study 102 (Part C subjects) tofersen treated period	ABCL: Overall Studies 101 and 102 (Parts A, B and C subjects) tofersen treated period	
Number of subjects with treatment emergent event	Tofersen 100 mg, Pool RC1 (N=72) n (%)	Placebo Pool RC2 (N=36) n (%)	Tofersen 100 mg (N=104) n (%)	Total tofersen 100 mg Pool ABCL1 (N=147) n (%)	Total tofersen all doses Pool ABCL2 (N=166) n (%)
Any event	69 (95.8)	34 (94.4)	103 (99.0)	146 (99.3)	164 (98.8)
CTCAE grade (a,d)					
Grade 1	25 (34.7)	15 (41.7)	12 (11.5)	17 (11.6)	25 (15.1)
Grade 2	32 (44.4)	15 (41.7)	43 (41.3)	64 (43.5)	68 (41.0)
Grade 3	10 (13.9)	4 (11.1)	25 (24.0)	36 (24.5)	39 (23.5)
Grade 4	N	N	5 (4.8)	7 (4.8)	7 (4.2)
Grade 5	N	N	18 (17.3)	22 (15.0)	25 (15.1)
Related event (b)	28 (38.9)	2 (5.6)	66 (63.5)	98 (66.7)	105 (63.3)
Events related to lumbar puncture (c)	58 (80.6)	29 (80.6)	87 (83.7)	126 (85.7)	143 (86.1)
Serious event	13 (18.1)	5 (13.9)	48 (46.2)	65 (44.2)	71 (42.8)

Related serious event	N	N	7 (6.7)	10 (6.8)	11 (6.6)
Events leading to drug withdrawal	N	N	23 (22.1)	30 (20.4)	3 31 (18.7)
Events leading to study withdrawal	3 (4.2)	0	22 (21.2)	28 (19.0)	31 (18.7)
Events leading to drug interruption	3 (4.2)	0	22 (21.2)	28 (19.0)	30 (18.1)
Events leading to hospitalisation	13 (18.1)	4 (11.1)	41 (39.4)	55 (37.4)	55 (33.1)
Number of subjects who died	1 (1.4)	0	18 (17.3)	22 (15.0)	25 (15.1)

NOTE 1: A subject can appear in more than one category.
(a) Each subject counted once at maximum CTCAE grade.
(b) Related as assessed by the investigator.
(c) Related to lumbar puncture as assessed by the investigator.
N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

The most common treatment-emergent adverse events (TEAEs) by preferred term (PT) (occurring in ≥ 10% subjects in either study 101 Part C or the total tofersen 100 mg population) are presented in Table 36.

Table 36: Adverse events by preferred term occurring in 10% or more subjects in any pooled group – data presented for Pool RC and ABCL1 – safety population (as of 15 Jul 2022)

	RC: 233AS101 Part C **(Part C participants) placebo-controlled period**		**ABCL1: Overall 233AS101 and 233AS102 (Parts A, B and C participants) tofersen-treated period**
	tofersen 100 mg (N=72) n (%)	**placebo (N=36) n (%)**	**Total tofersen 100 mg (N=147) n (%)**
Number of subjects with any event	69 (95.8)	34 (94.4)	145 (98.6)
PT			
Headache	33 (45.8)	16 (44.4)	90 (61.2%)
Procedural pain	41 (56.9)	21 (58.3)	84 (57.1)
Fall	17 (23.6)	15 (41.7)	66 (44.9)
Back pain	14 (19.4)	2 (5.6)	62 (42.2)
Pain in extremity	19 (26.4)	6 (16.7)	58 (39.5)
Arthralgia	10 (13.9)	2 (5.6)	47 (32.0)
Fatigue	12 (16.7)	2 (5.6)	39 (26.5)
Post lumbar puncture syndrome	13 (18.1)	11 (30.6)	34 (23.1)
CSF protein increased	6 (8.3)	1 (2.8)	36 (24.5)

	RC: 233AS101 Part C (Part C participants) placebo-controlled period		ABCL1: Overall 233AS101 and 233AS102 (Parts A, B and C participants) tofersen-treated period
	tofersen 100 mg (N=72) n (%)	placebo (N=36) n (%)	Total tofersen 100 mg (N=147) n (%)
Nausea	9 (12.5)	6 (16.7)	34 (23.1)
COVID-19	1 (1.4)	1 (2.8)	31 (21.1)
Myalgia	10 (13.9)	2 (5.6)	28 (19.0)
CSF white blood cell count increased	N	N	24 (16.3)
Contusion	3 (4.2)	1 (2.8)	24 (16.3)
Muscle spasms	5 (6.9)	2 (5.6)	27 (18.4)
Dizziness	4 (5.6)	3 (8.3)	26 (17.7)
Constipation	6 (8.3)	4 (11.1)	26 (17.7)
Nasopharyngitis	2 (2.8)	7 (19.4)	24 (16.3)
Pyrexia	3 (4.2)	1 (2.8)	23 (15.6)
Dyspnoea	4 (5.6)	5 (13.9)	19 (12.9)
Respiratory failure	N	N	20 (13.6)
Upper respiratory tract infection	5 (6.9)	2 (5.6)	15 (10.2)
Muscular weakness	4 (5.6)	4 (11.1)	19 (12.9)
Urinary tract infection	2 (2.8)	2 (5.6)	17 (11.6)
Diarrhoea	1 (1.4)	5 (13.9)	16 (10.9)
Salivary hypersecretion	4 (5.6)	1 (2.8)	15 (10.2)
Pleocytosis	N	N	13 (8.8)
Pain	N	N	15 (10.2)
Paraesthesia	6 (8.3)	6 (16.7)	14 (9.5)
Neck pain	4 (5.6)	4 (11.1)	12 (8.2)
Pneumonia aspiration	N	N	12 (8.2)
Post procedural complication	3 (4.2)	4 (11.1)	6 (4.1)

N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

TEAEs considered treatment related by the investigator

In Study 101 Part C, related AEs with an incidence of ≥5% in tofersen-treated participants were undisclosed information to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102.

In the tofersen 100 mg-treated group (ABCL1), related AEs with an incidence of ≥5% (as of 28 Feb 2023) were CSF protein increased (22.4%), pain in extremity (17.7%), CSF white blood cell count

increased (14.3%), headache (13.6%), myalgia (10.2%), pleocytosis (8.2%), procedural pain (6.8%), paraesthesia and back pain (6.1% each), and fatigue (5.4%).

The approach to identify ADRs used by the applicant took into consideration differences in the AE incidence to placebo from study 101 Part C, using a >5% threshold and causality assessment including risk factors/confounders, alternative explanations and general biological plausibility. Medical review of additional AEs from integrated study 101 and study 102 safety data complemented this assessment to identify other potential ADRs, based on events that may be related to nonclinical findings, on investigator evaluation of relatedness to study treatment or the LP procedure, on timing of events relative to drug exposure and the presence of a positive rechallenge.

The ADRs identified in the CDP for tofersen with incidences derived from Pool ABCL1 are given in Table 37.

Table 37: Adverse reactions for pooled group ABCL1 – safety population (cut-off 28 Feb 2023)

ADRs (PTs)	Frequency of ADRs n/N (%)	Frequency Category (a)
Pain	97/147 (66.0)	Very common
Fatigue	42/147 (28.6)	Very common
Arthralgia	50/147 (34.0)	Very common
Myalgia	28/147 (19.0)	Very common
CSF white blood cell increased	39/147 (26.5)	Very common
CSF protein increased	39/147 (26.5)	Very common
Pyrexia	27/147 (18.4)	Very common
Musculoskeletal stiffness	10/147 (6.8)	Common
Neuralgia	7/147 (4 . 8)	Common
Myelitis	4/147 (2 . 7)	Common
Radiculitis	4/147 (2 . 7)	Common
Aseptic meningitis	6/147 (4 . 1)	Common
Papilloedema	7/147 (4 . 8)	Common

NOTE 1: Myelitis includes preferred term of myelitis, myelitis transverse and neurosarcoidosis. Radiculitis includes preferred term of radiculopathy and lumbar radiculopathy. Aseptic meningitis includes preferred term of meningitis chemical, meningitis aseptic. Papilloedema includes preferred term of papilloedema, intracranial pressure increased.
NOTE 2: Pain includes preferred term of pain, back pain, and pain in extremity.
NOTE 3: CSF white blood cell increased includes preferred term of CSF white blood cell count increased and Pleocytosis.

Cerebrospinal fluid (CSF) findings

TEAEs related to CSF abnormalities

In Study 101 Part C, 12 patients (16.7%) in the tofersen 100 mg-treated group had AEs related to CSF laboratory abnormalities compared to 1 participant (2.8%) in the placebo-treated group. AEs (PT) related to abnormal CSF laboratory parameters reported in >4% of tofersen-treated participants were CSF protein increased, CSF white blood cell count increased, and pleocytosis. The AE related to abnormal CSF laboratory parameters reported in the 1 participant receiving placebo was undisclosed information to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102. .

In the integrated dataset of tofersen 100 mg-treated participants (ABCL1), 26.5% patients each had AEs of CSF white blood cell increased (including the PTs CSF white blood cell count increased and pleocytosis) and CSF protein increased. Many of the events of CSF white blood cell count increased and CSF protein increased in the tofersen 100 mg-treated participants (ABCL1) were assessed by the Investigator as treatment related.

CSF laboratory results

In Study 101 Part C, 78.3% tofersen vs. 25.0% placebo subjects had a shift to high CSF leukocytes, and 67.4% tofersen vs. 30.0% placebo subjects had a shift to high CSF protein. As of 28 Feb 2023, a total of 90.8% and 90.6% of the participants who received tofersen 100 mg (Pool ABCL1) had shifts to high

in their CSF leukocytes and CSF protein, with nearly all participants (91.2%) having at least 1 CSF leukocytes value >5 × 10^6/L and 80.3 % of participants having at least 1 value >10 × 10^6/L.

In pool ABCL1 an increase in mean CSF leukocytes and mean CSF protein was found, which appeared to stabilize each with continued tofersen, i.e. at above 20 leukocytes x 10^6/L after approx. 425 days and at approx. 1000 mg protein/L after approx. 500 days, respectively (analysis performed as of 15 Jul 2022).

Adverse Events that occurred within 24 hours of dosing

In study 101 Part C, the AEs of pain in extremity (15.4% vs. 8.3%), back pain (13.9% vs. 2.8%), myalgia (8.3% vs. 2.8%), and fatigue (12.5% vs. 2.8%) more commonly occurred within 24 hours in the tofersen 100 mg treatment group than in the placebo group (≥5% difference between groups).

90 Day intervals

Evaluation of AEs over time by 90 Day intervals revealed no pattern in AEs or SAEs over time and no indication for an increase in specific AEs over time, respectively (as of 15 Jul2022).

Adverse events of special interest (AESIs)

The following safety topics were considered of interest by the applicant (although not explicitly prespecified as AESIs in the study protocols).

• Since tofersen is administered intrathecally via LP, an investigator assessment of relatedness of AEs to the LP procedure was performed.

In study 101 Part C, 80.6% of participants each in the tofersen and placebo groups reported at least one AE assessed as related to the LP by the investigator. The most common AEs with an incidence of ≥5% in tofersen-treated participants were procedural pain, post lumbar puncture syndrome, headache, back pain, pain in extremity, nausea, and CSF white blood cell count increased. According to the submitted data, LP related AEs, that occurred with a higher frequency in tofersen vs. placebo subjects (and in at least 2 tofersen subjects) were: undisclosed information to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102. In the tofersen 100 mg-treated group (Pool ABCL1), 85.7% of the participants reported AEs that were considered related to the LP by the investigator. LP related AEs with an incidence of ≥10% were procedural pain (57.8%), headache (44.9%), back pain (32.7%) and post lumbar puncture syndrome (24.5%).

• Because thrombocytopenia, coagulation abnormalities, and renal toxicity have been observed with other subcutaneously or intravenously administered ASOs, the applicant reviewed all AEs reported in the clinical program to identify any relevant AEs for the original submission.

Thrombocytopenia

In the CDP, no AEs of thrombocytopenia or any cases of sustained or severe thrombocytopenia were identified in tofersen-treated participants and 6 participants (4.1%) in pool ABCL1 experienced shifts to low platelet count (as of 28 Feb 2023). In study 101 Part C, there was no difference in platelet counts over time between the placebo and tofersen-treated participants. The proportion of participants with shifts from normal or high platelet count at baseline to low value during treatment was similar in the tofersen and placebo group. As of 15 Jul 2022, across studies 101 and 102, 2 participants had a platelet value below 100 × 10^9/L (1 participant from study 101 Part B and 1 participant from study 101 Part C) and both had a repeat result within close proximity to the abnormal result that showed normal platelet counts. No bleeding events were reported during these transient episodes, and none of the low platelet values in the clinical studies were reported as AEs. Resolution of low platelet counts were observed.

Coagulation abnormalities

There was a high incidence of abnormal coagulation test results in all treatment groups across studies. The central laboratory frequently reported results outside of their laboratory normal ranges, while local laboratories of multiple study sites reported normal results. In addition, abnormal coagulation values were queried by the medical monitors and were confirmed to be considered not clinically significant. An investigation of the issue led to corrective actions. All results collected in the electronic case record forms were included in the analysis tables for coagulation, including shift tables.

Overall, treatment with tofersen was not associated with any clinically meaningful changes or percent changes over time for coagulation parameters, and the values were similar to placebo in study 101 Part C. A shift from baseline to high values was measured for activated partial thromboplastin time (aPTT) in 19.4% tofersen vs. 28.6% placebo subjects, for INR in 31.3% tofersen vs. 19.4% placebo subjects, and for PT (prothrombin time) in 50.0% tofersen and 51.9% placebo subjects, respectively. As of 28 Feb 2023, a total of 4 participants had coagulation panel-related AEs in the Investigations SOC reported: aPTT prolonged in 2, prothrombin time prolonged in one, and coagulation test abnormal in one patient. One event of aPTT prolonged in a tofersen treated participant was assessed as treatment related by the Investigator. All of these AEs were mild, self-limiting, and none were treatment limiting. None of these AEs was reported during the placebo-controlled study 101 part C.

Renal toxicity

In study 101 part C, patterns of creatinine and blood urea nitrogen (BUN) over time were similar between groups, shifts to high creatinine occurred in 1.4% tofersen versus 5.9% placebo subjects and shifts to high BUN values occurred in 23.5% tofersen versus 28.1% placebo subjects. However, AEs related to abnormal renal function or abnormal renal laboratory values were infrequent in both groups at a rate of <3%. There have been no reports of glomerulonephritis or nephrotic syndrome or other relevant renal toxicity in participants exposed to tofersen.

• Given tofersen's mechanism of action, there exists the hypothetical potential for adverse effects attributable to SOD1 deficiency; however, a "toxic" threshold for reduced SOD1 protein and/or SOD1 enzymatic activity has not been identified. To date, findings in nonclinical and clinical studies of tofersen do not clearly indicate any AEs attributable to SOD1 deficiency.

2.6.8.3. Serious adverse event/deaths/other significant events

Deaths

Overall, 25 tofersen treated subjects died during the CDP, no death has been assessed as treatment related by the investigator (as of 28 Feb 2023). During the controlled study parts, no death occurred in study 101 part A; in study 101 part B, 1/12 (8.3%) placebo subjects vs. 2/38 (5.6%) tofersen subjects died; in study 101 part C 1/72 (1.4%) tofersen subjects vs. 0/36 placebo subjects died.

In Study 101 Part B, 1 participant in the tofersen 20mg group with a history of obesity and sleep apnoea died due to an event of cardiovascular disorder, and 1 participant in the tofersen 60mg group died due to respiratory failure secondary to ALS.

In Study 101 Part C, 1 participant with a past medical history including longstanding coronary artery disease and other comorbidities died due to cardiac failure congestive (Day 114).

As of 15 Jul 2022, in Study 102, 12 participants died due to respiratory failure (among whom 1 participant also had a fatal event of ALS) and 2 died of respiratory arrest which were assessed as associated with ALS disease progression. Some of these events were associated with either the voluntary withdrawal of non-invasive ventilation or the participant declining to initiate noninvasive ventilation. Other deaths occurred in participants that had been dependent on noninvasive ventilation for several months and/or

were receiving hospice or palliative care due to advanced ALS disease. One additional participant died due to pneumonia aspiration.
One participant each in Study 102 died due to fatal events of sudden death and cardiac arrest. Both participants had a history of coronary artery disease, prior myocardial infarction, and other medical comorbidities. One participant each died due to fatal events of ALS and euthanasia. Finally, 1 participant died due to a fatal event of septic shock. The participant had additional SAEs, specifically acute respiratory distress syndrome, encephalopathy, pneumonia aspiration, pulmonary embolism, and respiratory failure, all starting on Study Day 908 with death on Study Day 917 in Study 102. Septic shock was suspected to be secondary to a polymicrobial infection. Three further participants of study 102 died between 15 Jul 2022 and 28 Feb 2023, with fatal AEs of respiratory failure, cardio-respiratory arrest and pneumonia aspiration in one subject each, none of which was considered related to tofersen.

SAEs
In study 101 part C, the incidence of SAEs was higher in the tofersen group (13/72 participants [18.1%]) compared with the placebo group (5/36 participants [13.9%]). SAEs were most frequently reported (≥ 4%) in the SOCs respiratory, thoracic and mediastinal disorders (5/72 participants [6.9%] in the tofersen group and 4/36 participants [11.1%] in the placebo group); general disorders and administration site conditions undisclosed information to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102; and nervous system disorders undisclosed information to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102.

SAEs considered related to study drug by the investigator are presented in Table 38.

Table 38: Related serious adverse events by system organ class and preferred term - safety population (cut-off: 28 Feb 2023)

	233AS101 Part C (Part C subjects) placebo-controlled period		Overall 233AS101 and 233AS102 (Parts A, B and C subjects) tofersen treated period	
	Tofersen 100 mg, Pool RC1 (N=72) n (%)	Placebo Pool RC2 (N=36) n (%)	Total tofersen 100 mg Pool ABCL1 (N=147) n (%)	Total tofersen all doses Pool ABCL2 (N=166) n (%)
Number of subjects with any related serious event	N	N	10 (6.8)	11 (6.6)
Nervous system disorders	N	N	7 (4.8)	6 (3.6)
Intracranial pressure increased	0	0	3 (2.0)	3 (1.8)
Headache	0	0	1 (0.7)	1 (0.6)
Lumbar radiculopathy	N	N	1 (0.7)	1 (0.6)
Myelitis transverse	N0	N	1 (0.7)	1 (0.6)
Radiculopathy		0	1 (0.7)	1 (0.6)
Infections and infestations	N	N	3 (2.0)	3 (1.8)

	233AS101 Part C (Part C subjects) placebo-controlled period		Overall 233AS101 and 233AS102 (Parts A, B and C subjects) tofersen treated period	
	Tofersen 100 mg, Pool RC1 (N=72) n (%)	Placebo Pool RC2 (N=36) n (%)	Total tofersen 100 mg Pool ABCL1 (N=147) n (%)	Total tofersen all doses Pool ABCL2 (N=166) n (%)
Myelitis	N	N	2 (1.4)	2 (1.2)
Meningitis aseptic	0	0	1 (0.7)	1 (0.6)
Eye disorders	0	0	1 (0.7)	1 (0.6)
Papilloedema	0	0	1 (0.7)	1 (0.6)
Gastrointestinal disorders	0	0	1 (0.7)	1 (0.6)
Gastritis	0	0	1 (0.7)	1 (0.6)
Pancreatitis	0	0	1 (0.7)	1 (0.6)
Injury, poisoning and procedural complications	NN	NN	1 (0.7)	1 (0.6)
Meningitis chemical			1 (0.7)	1 (0.6)
Investigations	0	0	0	1 (0.6)
CSF protein increase	0	0	0	1 (0.6)
CSF WBC count increased	0	0	0	1 (0.6)

NOTE 1: Only treatment emergent adverse events are summarized. A subject was counted only once within each system organ class and preferred term (MedDRA version 25.1).
NOTE 2: System organ class and preferred term are presented in decreasing frequency of the table's rightmost column.
NOTE 3: Relationship to study drug per investigator assessment.
WBC: white blood cell
N: Numbers removed to avoid unblinding of treatment allocation from Study 101 in the context of the ongoing open-label extension Study 102, as per the Unblinding Plan.

Additional information on related SAEs:

Myelitis and radiculitis

According to the referenced publication by Li 2021, studies have reported overall incidence rates of transverse myelitis ranging from 0.4 to 4.6 per 100,000 persons per year. A Canadian study (Klein 2010) reporting a higher incidence of 18.3 cases per 100,000 persons per year in Albuquerque may be an outlier.

A total of 6 participants experienced SAEs of myelitis or radiculitis as of 28 Feb 2023. The Investigator considered the events related to tofersen in 5 of 6 cases. Four participants reported SAEs of myelitis (PTs myelitis, myelitis transverse, and neurosarcoidosis), and 2 participants reported SAEs of radiculitis (PTs radiculopathy and lumbar radiculopathy). The number of doses of tofersen that a participant received prior to SAE onset varied between 1 and 24, and each SAE was associated with a CSF pleocytosis and elevated CSF protein.

Cases involving events consistent with myelitis are briefly summarized below.

• One SAE of neurosarcoidosis (verbatim term "neurosarcoid transverse myelitis") occurred in a participant with a comorbid history of sarcoidosis and was assessed by the Investigator as unrelated to tofersen. This participant continued treatment with tofersen while receiving immunomodulatory therapy for their sarcoidosis. The participant additionally reported a nonserious AE of thoracic radiculopathy which was assessed by the Investigator as mild and unrelated to study treatment.

• One participant with an event of myelitis, developed bilateral lower extremity numbness and weakness, MRI demonstrated extensive T2-hyperintense lesions and associated enhancement and CSF pleocytosis and elevated CSF protein was found. This participant required immunomodulatory therapy and rehabilitation, and tofersen treatment was discontinued. The event resolved without sequelae after approximately 3 months.

• One event of myelitis was asymptomatic; diagnosis was based on MRI contrast enhancement in the lower thoracic medulla and conus medullaris as well as CSF pleocytosis and elevated CSF protein. The participant continued treatment with tofersen despite a persistent CSF pleocytosis and elevated CSF protein.

• One event of myelitis occurred in a participant with a preceding nonserious event of aseptic meningitis and a preceding serious event of intracranial hypertension. The myelitic lesion was found on an MRI of the spine without associated symptoms, and approximately one month later the lesion was diminished in size with disappearance of enhancement. Tofersen was temporarily discontinued.

The two SAEs of radiculitis are briefly summarized below.

• One participant with an event of lumbar radiculopathy had symptoms of back pain and gait abnormality secondary to sensory changes associated with a mild CSF pleocytosis and elevated CSF protein but unremarkable MRI. Symptoms related to this event resolved within days, the participant continued on tofersen. The participant had 3 subsequent transient non-serious AEs of lumbar radiculopathy, which were assessed by the Investigator as tofersen related; all resolved within days, and the participant remains in Study 102.

• One event of radiculopathy was associated with of symptoms of back and thigh pain and subjective and objective sensory changes in the bilateral lower extremities. MRI examination demonstrated cauda equina enhancement. The event was considered resolved after approximately 9 months with sequelae of bilateral foot sensory loss, and loss of ankle jerk reflexes.

• In addition, nonserious AEs of lumbar radiculopathy (2 participants), and radiculopathy (1 participant) have been reported in the clinical trials. These non-serious events were assessed as mild to moderate and treatment related by the Investigator and did not lead to tofersen discontinuation.

Papilloedema/increased intracranial pressure.

The incidence of papilloedema in the general population is reported as 0.9 per 100,000 in the US and 1.56 per 100,000 in the UK [Rigi 2015].

In the CDP, four SAEs involving elevated intracranial pressure and/or papilloedema occurred (3 events with PT intracranial pressure increased, 1 event with PT papilloedema). All 4 SAEs were considered related to treatment by the investigator and medical review demonstrated papilloedema as a feature in each of these SAEs. Additionally, 2 participants had preceding AEs or SAEs of aseptic meningitis which could have affected CSF outflow and thereby increased their risk of development of papilloedema. All AEs and SAEs related to papilloedema/intracranial pressure increased were reported in participants who received tofersen 100 mg; no such events were reported in the placebo group].

These four SAEs are described below:

• One SAE of intracranial pressure increased was reported in a participant, who additionally reported an SAE of myelitis. The participant developed headaches and deterioration in their eyesight, bilateral papilloedema, and an elevated LP CSF opening pressure of 38 cm H2O, CSF pleocytosis, and elevated CSF protein. Approximately 8 months after onset the papilloedema was markedly regressed, and headaches and blurred vision had disappeared. As of the 28 Feb 2023 data cut, the event of intracranial pressure increased is ongoing, and treatment with tofersen remains ongoing. The most recent CSF opening pressure was + 25 cm H2O, and ophthalmological examination was normal with no papillary oedema.

• One SAE of intracranial pressure increased was reported after approximately 18 doses of tofersen, symptoms included headache, nausea, fever, photophobia, visual obscurations, and auditory distortions. Initial examination showed no papilloedema. LP was obtained and demonstrated an elevated CSF opening pressure, CSF pleocytosis, and elevated CSF protein. After initial improvement, symptoms recurred after a subsequent dose, and further evaluation showed papilloedema. The event was reported resolved after 27 days with sequelae (ongoing headache and visual disturbance). The participant continued to receive tofersen for approximately 6 months with doses alternating every other month and finally withdrew tofersen, because the patient still had some symptoms, he thought might be related to study drug.

• One SAE of papilloedema was reported after receiving 7 doses of tofersen 100 mg. Following a preceding SAE of aseptic meningitis treated with prednisone, the participant developed intermittent headache, visual acuity loss in the eye, peripheral visual field loss and visual floaters. Ophthalmological examination noted bilateral blind spot enlargement and optic nerve edema with hemorrhage as well as vitreous hemorrhage in the eye. The LP opening pressure was not elevated, and CSF pleocytosis and elevated CSF protein was found. The participant was started on acetazolamide, furosemide as well as IV methylprednisolone with tofersen dosing visits. The SAE of papilloedema was reported as resolved after 191 days, but Neuro-ophthalmology recommended continued IV methylprednisolone around study treatment infusions and continued acetazolamide. This participant additionally experienced another SAE as unrelated to study treatment but in the setting of treatment with acetazolamide and glucocorticoids as well as an SAE of headache assessed as rebound of intracranial hypertension associated with acetazolamide withdrawal.

• One SAE of intracranial pressured increased was reported after receiving 9 doses of tofersen 100 mg, symptoms included headaches and pulsatile tinnitus. LP demonstrated an elevated opening pressure, and prior CSF analyses found elevated CSF WBC and elevated CSF protein. 5 days after the SAE of intracranial pressured increased was reported, a coinciding nonserious, treatment related AE of papilloedema was reported, that resolved after 37 days. These events did not lead to study treatment interruption or withdrawal. Two to 3 days after beginning acetazolamide, the participant's headaches and pulsatile tinnitus resolved completely. The participant has continued treatment with acetazolamide, and the SAE was reported resolved after 247 days. As of 28 Feb 2023, the patient continued acetazolamide, and the most recent visual examinations noted VA 20/25+2 OD and 20/25 OS without disc edema, peripapillary hemorrhage, or pallor. A new nonserious, mild AE of intracranial pressure increased was reported then in this patient, that was ongoing as of 28 Feb 2023.

In addition, overall, 5 patients reported nonserious events of papilloedema. Of those, 3 participants reported only non-serious events of intracranial pressure increased (with or without additional AEs of papilloedema) and 2 participants reported nonserious AEs of papilloedema concurrent to serious AEs of papilledema/ intracranial pressure increased. In addition, one nonserious AE of "CSF pressure increased" assessed as tofersen related, mild and ongoing as of 28 Feb 2023 was reported.

Aseptic meningitis

Aseptic meningitis is seen at a rate of 2.9 to 7.6 cases per 100,000 persons in the general population [Mount and Boyle 2017].

During the CDP, two SAEs of aseptic meningitis (with the PTs meningitis chemical and meningitis aseptic, respectively in one subject each) were reported and are briefly described below:

• One participant with an SAE of meningitis chemical had symptoms of headache, neck pain, stiffness, and body aches, and a LP demonstrated CSF pleocytosis and elevated CSF protein but negative microbial studies. Study treatment was permanently discontinued, and the event resolved after 11 days.

• An SAE of aseptic meningitis occurred in one patient who also had an SAE of papilloedema. Symptoms included headache, neck stiffness, nausea, visual disturbances, and episodes of confusion. LP demonstrated a CSF pleocytosis and elevated protein but negative microbial studies. The participant was treated symptomatically with NSAIDS, steroids, and Benadryl; tofersen dosage was maintained, and the event resolved after 25 days.

Further review of clinical trial data identified 4 participants reporting nonserious AEs of aseptic meningitis with PTs of meningitis chemical (2) and meningitis aseptic (2), all of which were assessed by the Investigator as tofersen related and remained ongoing as of 28 Feb 2023. Of those, one event was previously reported with a VT of meningitis and the study site updated the event of VT to meningitis aseptic after query from the applicant. Two of the nonserious events of meningitis occurred in participants with AEs/SAEs of intracranial pressure increased/papilloedema.

In addition to the PTs included in the event of aseptic meningitis, there was 1 nonserious AE of meningism (resolved). This event was mild and study treatment related.

SAEs of gastritis and pancreatitis were reported in a single participant during study 102. . The SAEs of gastritis and pancreatitis were assessed as related to tofersen by the investigator given no alternative explanation. However, in reviewing AEs for the total tofersen population at all doses (ABCL2), no pattern of similar AEs was identified, and there were no other AEs of pancreatitis and no AEs in the Investigations SOC of elevated amylase and lipase. Two participants in Study 101 Part C reported AEs of gastritis, and 1 additional participant in Study 102 reported a mild AE of gastritis that was assessed as unrelated.

2.6.8.4. Laboratory findings

Haematology

In the integrated safety data set, there were no significant trends appreciated in hematology values, including hemoglobin, haematocrit, white blood cells, neutrophils, and lymphocytes. As of 28 Feb 2023, in pool ABCL1, AEs reported in the Investigations SOC or the Blood and lymphatic system disorders SOC that were related to hematology results were infrequent at rates of ≤3% for each PT, none of these AEs was assessed as tofersen related by the investigator.

Chemistry

Hepatic function (as of 15 Jul 2022 unless indicated otherwise)

In study 101 part C, treatment with tofersen was not associated with any clinically meaningful changes or percent changes over time in liver function tests (LFT), and the values were similar to those for placebo. Shifts to high alanine transaminase (ALT) were less frequent in tofersen 100 mg than in placebo-treated participants (34.8% vs. 44.0%) but shifts to high aspartate transaminase (AST) (30.4% vs. 21.9%), alkaline phosphatase (7.1% vs. 2.8%), and gamma-glutamyl transferase (GGT) (12.6% vs. 5.9%) were more frequent in tofersen 100 mg-treated participants than in placebo-treated participants.

There were no Hy's Law cases in the integrated safety set.

Study 101 Part C had 1 participant with an ALT > 5 x the upper limit of normal (ULN), 1 participant with an AST > 3x ULN, 1 participant with a total bilirubin > 1× (≤ 1.5 x) ULN, and 1 participant with an alkaline phosphatase > 1.5x ULN. In all other instances, changes in liver function parameters, including bilirubin, were mild and generally similar between the tofersen 100 mg-treated and placebo-treated participants. In the overall tofersen 100 mg-treated participants (ABCL1), 5.4% of participants had AST > 3 x ULN, and 10.9% had ALT > 3 x ULN.

As of 28 Feb 2023, in all tofersen 100mg treated participants, AEs related to LFT parameters in the SOC Investigations of ALT increased (5.4%), AST increased (4.1%), hepatic enzyme increased (4.1%), GGT increased (1.4 %), transaminases increased (1.4 %), blood alkaline phosphatase increased (1.4%), and liver function test abnormal (0.7%) each occurred in <6% of participants. According to ISS Addendum 2023, Appendix 2.3, one event each of ALT increased, AST increased, and blood alkaline phosphatase increased, respectively was considered treatment related by the investigator. AEs in the SOC Hepatobiliary disorders were reported in ≤ 6 % of tofersen 100 mg-treated participants and included PTs of hepatic steatosis, cholelithiasis, cholecystitis, bile duct stone, hepatic function abnormal, and hypertransaminasaemia; none of these events were assessed by the Investigator as related to study treatment.

The following hepatic and biliary laboratory abnormalities of note are presented in the SCS:

• One participant had events of increased ALT and AST that were assessed by the Investigator as related to study treatment and mild in severity. This participant used confounding concomitant medication and LFT values fluctuated throughout the study including baseline abnormalities and frequent normalisation despite uninterrupted study treatment dosing.

• One participant in study 101 Part C had events of ALT increased and AST increased which were grade 3 severity and assessed as unrelated to study treatment. This participant had an elevated ALT (1.7 x ULN) and AST (1.1 x ULN) on Day 1 which on Day 15 increased to ALT 5.2 x ULN and AST (3.7 x ULN), corresponding with the time that the AEs were reported (Day 14). While the AEs did not resolve over the course of the study, Day 29 values improved to ALT 3 x ULN and AST 1.1 x ULN and remained <1.5 x ULN for ALT and <1.6 x ULN for AST for the remainder of their participation in Study 101 Part C. ALT in Study 102 ranged from approximately 1.2 x ULN to 2.0 x ULN, while AST was largely within normal limits.

• One participant in study 101 Part C and the LTE experienced an AE of blood alkaline phosphatase increased, which was assessed as study treatment related. This participant had no other abnormal LFT parameters throughout the study. This AE was assessed as moderate in severity, led to study treatment interruption, and was not resolved as of the 15 July 2022 data cut. AP levels were normal throughout study 101 Part C and before Week 60 in the LTE, when the value was 1.8203 µkat/L (<1.04 x ULN); it reached 1.3 x ULN at Week 80, and 3.3 x ULN at Week 104, leading to tofersen withdrawal. AP levels remained stably elevated thereafter despite no tofersen administration since approx. 11 months (as of 28 Feb 2023). No other study participants have reported a related AE of blood alkaline phosphatase increased.

Urinalysis

As of 15 Jul 2022, treatment with tofersen was not associated with any clinically meaningful changes over time in urinalysis parameters and there were no clear patterns of treatment-emergent abnormalities in urinalysis. In the SOC Investigations, AEs of blood urine present, cells in urine, protein urine, protein urine present, white blood cells urine positive, glucose urine present, and nitrite urine present each occurred in 1 subject of all tofersen 100 mg-treated participants (ABCL1). In the SOC Renal and urinary disorders, AEs of undisclosed AE (undisclosed to avoid unblinding of treatment allocation from Study 101

in the context of the ongoing open label extension Study 102) and haematuria each occurred in ≤2% of all tofersen 100 mg-treated participants (ABCL1). None of these AEs were assessed as treatment related by the Investigator.

Vital signs

Overall, treatment with tofersen was not associated with changes over time for the vital sign measurements (body temperature, heart rate, blood pressure, weight and respiratory rate) during study 101 part C and study 102 (according to the Interim 2 Report of study 102, cut-off date: 16 Jan 2022). In study 101 Part C, weight gain ≥ 7% occurred at an increased incidence in the tofersen vs. the placebo group (14.3% vs. 5.6%), while weight loss occurred at a lower incidence in the tofersen vs. the placebo group (12.9% vs. 19.4%). Across pool RC1 and ABLC1 a similar proportion of subjects experienced weight gain and weight loss, respectively. Apart from pyrexia and related PTs of chills, feeling hot, and feeling cold, respectively AEs of vital sign abnormalities were infrequent, and (apart from temperature related AEs) none were assessed by the Investigator as related to tofersen (as of 15 Jul 2022).

Overall, treatment with tofersen was not associated with changes over time for the electrocardiogram (ECG) measurements (according to the Report of study 101 part C and the Interim 2 Report of study 102, cut-off date: 16 Jan 2022). In study 101 part C, a higher incidence of increase in QTc >30 ms to 60 ms was found in tofersen vs. placebo subjects (11.3% vs. 5.6%). An increase in QTc > 60 ms, or QTc values > 480 ms or > 500 ms was not reported in this study part and was each reported in single subjects only of the total tofersen 100 mg population (pool ABCL1, as of 15 Jul 2022). As of 28 Feb 2023, in pool ABCL1, AEs reported in the cardiac disorders SOC and in the investigations SOC related to ECG abnormalities, respectively were generally infrequent (<4% by PT) and were all considered unrelated to treatment. AEs were further evaluated for events consistent with potential pro-arrhythmic effects using the MedDRA Standardized Medical Dictionary for Regulatory Activities Query (SMQ) "Torsade de pointes/QT Prolongation" and PT "Seizure." During study 101 part C, respective events were reported in 1 (1.4%) tofersen and 2 (5.6%) placebo subjects.

In study 101 part C, shifts in ECG to abnormal, clinically significant AE values were low and similar between the tofersen and placebo group.

In study 101 Part C, treatment-emergent suicidal ideation occurred at similar rates between the tofersen (6.9%) and placebo-treated participants (5.6%) and suicidal behaviour did not occur (measured by C-SSRS). There were no clinically significant differences or trends in the Mini-Mental State Exam between the tofersen 100 mg-treated participants (RC1) and the placebo participants (RC2) in Study 101 Part C.

2.6.8.5. Safety in special populations

Intrinsic and extrinsic factors (as of 15 Jul 2022):

Disease progression status

The most common AEs in participants in the "enriched" subgroup of SOD1-ALS participants (i.e., deemed most likely to have faster disease progression based on the original protocol defined enrichment definition) were generally similar to those in the "other" subgroup of SOD1-ALS participants. Participants in the "enriched" subgroup were noted to have a higher frequency of AEs ≥ Common Terminology Criteria for Adverse Events (CTCAE) grade 3. Within the "enriched" subgroup, the incidence of SAEs was higher in the tofersen group (11/39 participants [28.2%]) compared with the placebo group (4/21 participants [19.0%]) whereas SAEs in the "other" subgroup occurred less frequently (in 2/33 [6.1%] tofersen vs. 1/15 [6.7%] placebo subjects). In particular, participants in the "enriched" subgroup who received either tofersen or placebo in Study 101 had more events in the SOC of Respiratory, thoracic, and mediastinal

disorders than did participants in the "other" subgroup. Of note, serious neurologic events occurred in participants in both the "enriched" and the "other" subgroup.

Age

The total tofersen population in both the RC and ABCL1 pools ranged in age from 23 to 78 years. Study 101 part C included nine tofersen and 5 placebo patients ≥ 65 years, and 20 (13.6%) patients of pool ABCL1 were ≥ 65 years. Evaluation of the overall summary of AEs showed that in the RC and ABCL1 pools, both subgroups (<65 and ≥65 years) generally had similar proportions of participants experiencing AEs in each AE category. Review of individual AEs (incidence ≥ 5% in the RC pool and ABCL1 groups) showed that the incidence and type of AEs for tofersen versus placebo subjects in study 101 part C and in the ABCL1 pool within both age subgroups were generally consistent with those of the overall population and did not reveal any notable concerns in any subgroup.

Sex

In the RC and ABCL1 pools, there were more male (57.4% and 55.8%, respectively) than female participants. In the overall summary of AEs, both subgroups had similar proportions of participants experiencing AEs in each AE category. Review of individual AEs (incidence ≥ 5% in the RC and ABCL1 groups) showed that the incidence and type of AEs were generally consistent with those of the overall population and did not reveal any notable concerns in either subgroup.

Race

Most participants in studies 101 and 102 were white, or race was not reported (e.g. in Pool ABCL1 the respective numbers were 59.9% and 30.6% respectively), limiting the AE comparison across race.

Site of onset was specified as a subgroup of interest; however, only a small number of participants was enrolled in study 101 Part C with bulbar onset (3 tofersen, 3 placebo) compared to other regions of onset (69 tofersen, 33 placebo).

Participants with *hepatic or renal impairment* were not included in the clinical studies with tofersen.

Geographic region

Study 101 Part C included participants from North America (48 tofersen, 25 placebo), Europe (21 tofersen, 7 placebo), and Asia-Pacific (3 tofersen, 4 placebo). No differences were identified by the applicant when comparing AEs in participants who received tofersen 100 mg (RC1) against those receiving placebo (RC2) across geographical regions, and review of all participants who received tofersen 100 mg (group ABCL1) did not identify any regional differences in commonly reported AEs or serious neurological adverse events. However, according to ISS3 Appendix 2.3.2, Output 13, in the overall tofersen 100 mg population (pool ABCL1), CSF protein increased was reported as AE in 46.7% European vs. only 12.6% North American patients, and CSF WBC count increased was reported as AE 26.7% European vs. 11.6% North American patients.

There are no data from clinical studies on the use of tofersen during pregnancy and lactation.

2.6.8.6. Overdose and abuse potential

No cases of overdose associated with tofersen have been reported in clinical studies (as of 15 Jul 2022).

Given the targeted mechanism of action and intrathecal route of administration (necessitating in-clinic dosing), it is anticipated that tofersen would have a negligible potential for drug abuse. The dependence potential of tofersen has not been studied.

2.6.8.7. Immunological events

As of 15 Jul 2022, in the total 100 mg tofersen population (pool ABCL1), 93/147 (63.3%) subjects developed positive ADAs in plasma, and ADAs were found to be persistent in the majority of ADA positive subjects (76/93; 81.7%). Neutralizing ADAs or ADA status in CSF was not examined.

Impact of ADAs on PK

The presence of ADAs appeared to decrease plasma clearance by 32.2%.

Impact of ADAs on efficacy

According to the applicant, given the relatively small number of participants in each group and individual disease heterogeneity, no clear conclusions regarding the comparison of ALSFRS-R in participants with persistent positive ADAs and other participants can be drawn (and no comprehensive evaluation has been provided). However, the applicant expects no impact on efficacy, as large molecules such as ADAs have limited penetration through the blood brain barrier and ADAs were not found to be a significant covariate for either the SOD1 protein or NfL in the PK/PD models.

Impact of ADAs on safety (as of 15 July 2022, unless stated otherwise)

In order to explore the impact of tofersen ADA response on safety, the incidence of AEs selected by SMQs (of hypersensitivity, anaphylactic reaction, and angioedema) was evaluated by ADA status. In study 101 Part C, the frequency of AEs identified by these SMQs was lower in ADA positive tofersen subjects (22.7%) compared to both, ADA negative tofersen subjects (28.0%) and ADA negative placebo subjects (32.4%); one of 2 (50%) ADA positive placebo subjects had an AE in these SMQs (PT dyspnoea). In the total 100 mg of tofersen safety population (ABCL1), AEs identified by these SMQs were reported in 60 (64.5%) ADA positive and 31 (57.4%) ADA negative participants. As of 28 Feb 2023, 4 subjects in pool ABCL1 had multiple pyrexia events that were considered tofersen related by the investigator. Two of these patients had positive and two had negative ADA status.

As of 15 July 2022 data cut, no impact of tofersen ADA was observed on the incidence of serious neurological ADRs (myelitis, radiculitis, papilloedema, aseptic meningitis). In the overall tofersen 100 mg-treated group (ABCL1), 4 participants (2.7%) with serious neurologic ADRs had positive ADA status, while 6 (4.1%) had negative ADA status. No further serious neurological ADRs were reported between 15 July 2022 and 28 Feb 2023.

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

The DDI potential for ASOs is low. *In-vitro* studies in human biomaterials did not provide any indication for a potential DDI of tofersen with common drug metabolising enzymes or drug transporters. Therefore, no dedicated human DDI studies with tofersen have been performed.

2.6.8.9. Discontinuation due to adverse events

AEs leading to treatment discontinuation were reported in 4/72 (5.6%) tofersen vs. 0/36 placebo subjects in Study 101 part C. In the total tofersen 100 mg population, 30 reported AEs that led to study treatment discontinuation, most of which were associated with the underlying ALS disease (including the death cases). With regard to the identified serious ADR of tofersen, the events of myelitis, meningitis chemical, and neurosarcoidosis led to tofersen withdrawal in one subject each. Also, one SAE of intracranial pressure increased finally led to tofersen withdrawal.

AEs leading to study treatment interruption occurred in 28 of tofersen 100 mg-treated patients. Each AE (by PT) that led to study treatment interruption occurred in only 1 participant apart from AEs of COVID-

19 (in 10 participants) and deep vein thrombosis, nasopharyngitis and headache (in 2 participants each), respectively. Respective events (by PT), which occurred in single subjects only, included (amongst others), myelitis, papilloedema, back pain, procedural pain and post lumbar puncture syndrome.

2.6.8.10. Post marketing experience

N/A (Tofersen was approved by the FDA only after the safety data cut of 28 Feb 2023).

2.6.9. Discussion on clinical safety

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

As of the 28 Feb 2023, safety data are available from 166 ALS patients who received tofersen in the placebo controlled multi-part study 101 and open-extension study 102 and who were SOD1-ALS patients with the exception of 4 subjects in the single dose study 101 part A. Of these, 147 SOD1-ALS subjects have received at least 1 dose of 100 mg tofersen (the intended dose), 116 SOD1-ALS subjects have received 100 mg tofersen for at least one year and 95 subjects for at least two years. The maximum total duration of exposure for 100 mg (any dose of) tofersen is 245 (316) weeks. The number of overall tofersen exposed subjects and in particular the controlled exposure in study 101 part C which is pivotal for this application (i.e. 72 tofersen and 36 placebo subjects treated for 24 weeks) is rather limited, taking into consideration that the underlying disease can lead to a broad spectrum of symptoms including extra-motor symptoms, e.g. dysfunction of the autonomic system, cognitive and behavioural changes, and complications of malnutrition and restricted mobility, respectively. Therefore, TEAEs derived from uncontrolled ALS studies are often difficult to interpret with regard to causality. Nevertheless, taking into consideration that SOD1-ALS is an ultra-orphan disease, the safety exposure is considered sufficient for a benefit risk evaluation. As the general cut-off date for the safety data of the original submission was the 15 July 2022, updated safety data focusing on key safety analyses from ongoing long-term extension study 102 (including updated pooled safety analyses) as of 28 Feb 2023 were provided by the applicant in order to further inform long-term safety of tofersen.

The applicant's discussion of safety focuses on the comparison of safety in the active treatment group of 'pivotal' study 101 part C, receiving 100 mg tofersen (Pool RC1), with the respective placebo group (Pool RC2) as well as on Pool ABCL1, which includes all participants receiving 100 mg tofersen during study 101 and/or 102. This is considered appropriate, as 100 mg is the intended tofersen dose, constitutes the highest tofersen dose evaluated in the clinical studies and also the dose that the majority of patients received.

The vast majority (>94%) of participants across the tofersen and placebo group of study 101 part C reported at least one TEAE, many of which were consistent with ALS or the LP procedure. However, the overview of safety revealed a higher incidence of AEs considered treatment related by the investigator in tofersen vs. placebo treated subjects (38.9% vs. 5.6%) and the severity of TEAEs by CTCAE grading tended to be higher in the tofersen 100 mg compared to the placebo group. In line with this, SAEs occurred with a somewhat higher frequency in tofersen vs. placebo subjects (18.1% vs. 13.9%). During longer exposure 66.7% subjects treated with tofersen 100 mg experienced treatment related AEs (Pool ABCL1) and the incidence of SAEs assessed as related by investigator in the overall 100 mg tofersen treated subjects (Pool ABCL1) was 6.8%.

The approach used by the applicant to identify adverse drug reactions (ADRs) is generally considered acceptable and took into consideration differences to placebo in the AE incidence from the 'pivotal' study, using a >5% threshold based on the 2:1 randomisation and population size and causality assessment.

In addition, medical review of additional AEs from the integrated safety data from Study 101 and Study 102 was performed to identify other potential ADRs, based on events related to nonclinical findings, investigator assessment of relatedness to study treatment and the LP procedure, the timing of events and the presence of a positive rechallenge.

The most common identified ADRs are pain (including the PTs pain, back pain, and pain in extremity; reported in 66.0% subjects in the total tofersen Pool ABCL1), fatigue (28.6%), arthralgia (34.0%), myalgia (19.0%), and CSF abnormalities (white blood cell increased and protein increased, reported in 26.5% subjects each) and these were all identified based on a > 5% higher incidence in tofersen vs. placebo subjects in study 101 part C. In addition, pyrexia has been identified as an ADR due to positive rechallenge experiences in participants receiving tofersen and as pyrexia occurred in close proximity to timing of exposure in some subjects (i.e. pyrexia within 24 hours of tofersen administration occurred in 9.1% subjects in Pool ABCL1). Pyrexia was reported in 18.4% subjects in the total tofersen 100 mg population (Pool ABCL1), overall, no pyrexia event was severe or serious, and no increase in the incidence of pyrexia with longer treatment duration was recognizable. Pyrexia is an ADR that is shared by multiple ASO drugs and may be mediated by an immune response to the ASO. Overall, 70 AEs related to pyrexia, that were considered treatment related by the investigator, were reported in 8 participants in the clinical trial database. In half of these latter subjects (4/8), a positive rechallenge was found with 6 to 25 multiple events. Nevertheless, no increase in severity of pyrexia is recognizable, as all related pyrexia events were grade 1 in severity. Also, in all of the patients with a positive pyrexia rechallenge no action was taken with study treatment as a result of the event and no precautionary measures for tofersen related pyrexia (e.g., antipyretics) were taken around the time of any pyrexia events. As more than half of the ABCL1 population developed positive ADA titres, it is considered reassuring that of the patients with multiple tofersen related pyrexia events, only two had positive ADA titers, whereas two patients had negative ADA status.

Irrespective of causality, the incidence of headache was comparable in tofersen vs. placebo treated subjects during study 101 part C (45.8% vs. 44.4%). Further evaluation revealed, that only 3/147 (4.1 %) of these patients had headache events considered related to tofersen treatment but not to the LP. Two of these subjects had a confounding medical history, in none of these subjects the tofersen dose was changed due to the event. Irrespective of headache as a frequent event after LP procedures or as a possible symptom of the identified neuroinflammatory adverse reactions, a causal relationship of headache with tofersen cannot clearly be derived from the available data and separate labelling of headache as a tofersen related ADR is currently not warranted. Procedural pain was reported in more than half of the subjects of study 101 part C in both treatment groups (56.9% RC1 vs. 58.3% RC2). While lumbar punctures are frequently associated with procedural pain, further evaluation of the applicant did not allow for firm conclusions regarding a causal relationship between tofersen and procedural pain, e.g. via local irritation.

Analyses of TEAEs, that occurred ≥ 5% more frequently within 24 hours of dosing in the tofersen vs. placebo group of study 101 part C indicate, that the identified ADRs of pain in extremity, back pain, myalgia, and fatigue, respectively frequently occur within 24 hours of tofersen administration. It is stated in the Clinical overview, that an analysis of events reported within 24 hours after dosing was performed to examine possible acute toxicities and revealed no safety concerns. However, a rather small imbalance was also found with regard to pruritis reported within 24 hours of study drug administration. As of the 15 Jul 2023, in the overall tofersen 100 mg pool (ABCL1), eight participants reported 15 AEs related to pruritus, rash, rash pruritic, dermatitis, and/or blister occurring within 24 hours of tofersen administration. None of the 15 events was serious. Eight of these 15 AEs had no exact day of onset specified and, using a conservative approach, were considered of onset within 24 hours of tofersen dosing. Seven of the 15 events were considered related to treatment, however, none of these events let to tofersen discontinuation or dose modification. Overall, relevant AEs considered related to tofersen by

the investigator with known onset within 24 hours of tofersen occurred only in a single subject and concerned pruritus at different locations of the lower body after the second last dose before the data cut with mild severity each. A clear risk of tofersen for hypersensitivity reactions cannot be concluded at present. The applicant will continue to monitor for hypersensitivity reactions in the ongoing tofersen trial, EAP and in the post-marketing setting, which is endorsed.

It is considered reassuring, that analyses by 90 Day intervals revealed no apparent pattern in AEs or SAEs over time and no indication for an increase in specific AEs over time, respectively (as of 15 July 2022). Nevertheless, results need to be interpreted with caution due to considerably decreasing subject numbers with time in pool ABCL1.

Overall, as of 28 Feb 2023, 25 tofersen treated subjects died during the CDP, no death was assessed as treatment related by the investigator. During the controlled study parts, there was no clear imbalance in fatal events between tofersen and placebo treated subjects. From the information provided, the majority of deaths in tofersen treated subjects during the CDP was consistent with and considered related to the known course of ALS. As of the 15 Jul 2022, 14 of 22 fatal AEs were in the respiratory, thoracic and mediastinal disorders SOC, including 12 cases of respiratory failure and 2 cases of respiratory arrest which were associated with ALS disease progression. Other fatal AEs by PT attributable to the underlying disease, or consequences thereof, included pneumonia aspiration, ALS, euthanasia, and septic shock. Of note, regarding this latter case, the fatal event occurred more than one month after the last IMP administration and the narrative is not indicative of an infection due to the LP. The cause of a fatal event of "cardiopulmonary event" (PT) is also compatible with advanced ALS. Three subjects with fatal events of cardiac arrest, cardiac failure congestive, or sudden death, each had an increased risk of cardiac death based on their medical history and cardiovascular risk factors. From the available information on death cases, no safety signal arises. Three further participants of study 102 died between 15 Jul 2022 and 28 Feb 2023, with fatal AEs of respiratory failure, cardio-respiratory arrest and pneumonia aspiration in one subject each, none of which was considered related to tofersen.

Serious ADRs of myelitis and radiculitis, papilloedema and aseptic meningitis have been identified based on a higher incidence than would be expected in the study population, biological plausibility, and apart from papilloedema also correlation with non-clinical findings. All of these events occurred during the placebo-controlled study parts and were associated with CSF WBC and protein increase. These serious ADR are all consistent with nervous system inflammation that may be secondary to the proinflammatory effect of the ASO tofersen and may fall within the same spectrum of neuroinflammation, which is also supported by the finding, that some patients had more than one type of these serious ADRs. As of 28 Feb 2023, overall, 12 neuroinflammatory SAE were reported in 10 patients. However, although all of these serious ADR appear to involve the central nervous system (CNS), the neuroinflammation is not limited to the CNS in all events, as radiculitis would be considered a disorder of the peripheral nervous system.

Myelitis (including the PTs myelitis myelitis transverse, and neurosarcoidosis), radiculitis (including the PTs radiculopathy, and lumbar radiculopathy), papilloedema (including the PTs papilloedema, and intracranial pressure increased) and aseptic meningitis (including the PTs aseptic meningitis, and meningitis chemical) are each proposed to be given in SmPC section 4.8 as common adverse events, which is endorsed. The incidences have been clarified as requested.

Each of these serious ADRs was associated with CSF pleocytosis and increased CSF protein, however, with varying degrees. Since the vast majority of subjects in pool ABCL1 had CSF pleocytosis and increased CSF protein, these CSF abnormalities are considered of less value for identifying patients at risk for serious neurological ADRs. The requested evaluation performed by the applicant did not identify any risk factors for these events, including (but not limited to) no clear association with duration of exposure, ADA status or titre, or age. In three of the 12 neuroinflammatory SAEs reported in the clinical

studies, study treatment was interrupted, in two events study treatment was permanently discontinued. Two of the 12 neuroinflammatory SAEs resolved with sequelae: One event of intracranial pressure increased (ICP) had ongoing headache and visual disturbance, which finally led to study treatment withdrawal. One event of radiculopathy resolved with continued study treatment after approximately 9 months with sequelae of CSF pleocytosis, elevated CSF protein, bilateral foot sensory loss, and loss of ankle jerk reflexes. A further SAE with the PT ICP increased was reported not resolved as of 28 Feb 2023, however, the clinical symptoms and papillary oedema had disappeared, while the CSF opening pressure was 25 cm H2O as of the most current value reported in Table 1 of the applicant's Response; no action was taken with study treatment in this event. The other 9 neuroinflammatory SAEs resolved (without clinical sequelae) despite no action taken with study treatment in 5 of these events. In studies 101 and 102, overall, five patients with SAEs associated with myelitis and radiculitis, respectively had inflammatory findings in the spinal MRI. In two of these patients, study treatment was administered while inflammatory findings were present, in two subjects, study treatment was discontinued or only resumed after imaging was improved, respectively, in one subject it remains unclear, whether study treatment was administered while inflammatory imaging findings were present.

As there was high variability in manifestations, courses and required action in these SAE cases, the applicant's position not to make specific recommendations in the SmPC in case of neuroinflammatory ADRs can be agreed. Instead, diagnostic evaluation and clinical management should be performed according to standard of care and the consequences regarding tofersen treatment (i.e., treatment discontinuation, interruption or continuation) in case of neuro-inflammatory adverse reactions should be decided upon by the treating physician at the individual patient level.

All four patients with SAEs of papilloedema/increased ICP had typical symptoms such as headache or visual disturbance which are clinically monitorable. Therefore, routine measurement of the opening pressure is not generally recommended with tofersen treatment. Nevertheless, an evaluation of the fundus of the eye before any LP is considered standard.

Irrespective of tofersen or placebo administration, the majority of study subjects experienced LP related events, i.e. 80.6% in either treatment group of study 101 part C. While some of the events considered 'LP procedure related events' by the Investigators were reported only in the tofersen but not in the placebo group and have actually been identified as tofersen related events, established adverse events of an LP procedure (e.g. procedural pain, post-lumbar puncture syndrome, and headache) were frequently reported across both treatment groups, as expected, but are currently still not addressed in the proposed SmPC and should be implemented (see also attached documents). During the clinical study program, one event of propionibacterium infection of the CSF was reported, that was related to the LP. The event was moderate and non-serious, however required intravenous antibiotic treatment for two weeks. Limited data on the event are available, however, no AEs suggestive of infectious complications (e.g. no meningitis) were reported in this subject. Apart from this, no events suggestive of a serious CNS infection and no SAE of (CNS) infection due to the LP was reported in the CDP. SmPC Section 4.4 has been amended to warn about the risk of CNS infection (amongst other established AEs of an LP procedure) and lumbar puncture related adverse reactions are also adequately presented in section 4.8 of the SmPC.

In tofersen treated patients, an increase in mean cerebrospinal fluid (CSF) leukocytes and mean CSF protein was found, which appeared to stabilize at greater than 4 times the upper normal value for leukocytes (i.e. at above 20 leukocytes x 10^6/L after approx. 425 days) and at nearly twice the upper normal value for protein (i.e. at approx. 1000 mg protein/L after approx. 500 days) after approx. 500 days of continued tofersen exposure in each case. Although the vast majority of subjects in pool ABCL1 had upward shifts of CSF protein (90.6%) or leukocytes (90.8%), respectively, these were reported as TEAEs in less than half of the cases (as of 28 Feb 2023). While the serious neuroinflammatory ADRs were all associated with increased CSF leukocytes and protein, CSF abnormalities per se were not

treatment limiting. An increase in CSF white blood cell counts and CSF protein, respectively was also found in the 9-months non-human primate studies.

As of 15 Jul 2022, no clear safety signals arose from other laboratory evaluations. With the absence of findings as of this date the applicant did not pursue focused investigations in the updated safety data as of 28 Feb 2023. There may be a minor imbalance with regard to clinically significant abnormalities in LFT parameters in tofersen vs. placebo treated subjects in study 101 part C, however these concerned total bilirubin >1x ULN (but ≤ 1.5x ULN), ALT >5 x ULN, AST > 3x ULN and alkaline phosphatase > 1.5x ULN . Similarly, TEAEs, related to LFT parameters in the Investigations SOC occurred more commonly in tofersen subjects during study 101 part C. However, taking the overall data concerning LFT parameters into consideration, as well as low subject numbers and 2:1 randomisation, this may be due to a chance finding. In particular there were no meaningful mean or percent changes in LFT parameters over time, and across different LFT parameters there was no consistent higher incidences in shifts towards higher values in tofersen vs. placebo treated subjects. There were no cases of Hy's law, and apart from one SAE of cholecystitis (considered not related to treatment by Investigator), none of the AEs related to LFT parameters in the investigations SOC and of the AEs in the hepatobiliary disorders SOC was serious.

As of the 28 Feb 2023, the incidence of TEAEs related to LFT parameters in the investigations SOC as well as TEAEs in the hepatobiliary disorders SOC remained low and apart from one AE of mild AST and mild ALT increased as well as one moderate event of alkaline phosphatase increased, respectively, these were not considered related to treatment. Also, follow-up information and a causality assessment of these cases do not clearly indicate a causal relationship with tofersen. Ambiguities regarding LFT units were clarified and corrected during the procedure.

The available data derived from the tofersen CDP do not indicate a clear risk of thrombocytopenia, coagulation abnormality or bleeding, or renal toxicity. However, thrombocytopenia and coagulation abnormalities as well as renal toxicity have been observed after administration of some subcutaneously and intravenously administered ASOs. Based on class effects data, these risks can therefore not be excluded for tofersen and should be further followed. A respective warning has been added to SmPC section 4.4.

The significance of an imbalance found with regard to increases in QTc >30 to 60 ms in tofersen vs. placebo subjects (11.3% vs. 5.6%) in study 101 part C is unclear. There was no increase in relative to placebo control in the incidence of cardiac AEs associated with delayed ventricular repolarisation in tofersen treated patients. In routine ECG monitoring, an increase in QTc > 60 ms, or QTc values > 480 ms or > 500 ms was not reported in this study part and was each reported in single subjects only of the total tofersen 100 mg population, and no AEs associated with these outlier values were reported (pool ABCL1, as of 15 Jul 2022). Also, in pool ABCL1 (as of 28 Feb 2023), AEs reported in the cardiac disorders SOC and in the investigations SOC related to ECG abnormalities, respectively were generally infrequent (<3% by PT, apart from tachycardia reported in 3.4%) and were all considered unrelated to treatment. A hERG assay as well as the provided PK/PD modelling of QTc data from the clinical studies was not indicative of a proarrhythmic potential of tofersen, however the PK/PD modelling data are of limited value (as discussed in the Clinical pharmacology section).

Immunotoxicology has not been evaluated in the preclinical development program. However, it is acknowledged, that immune reactions in animals may be of questionable relevance for humans. As of 15 Jul 2022, approx. two thirds (63.3%) of the total tofersen 100 mg safety population (pool ABCL1) developed positive ADAs in plasma, the majority of which remained persistent (in 81.7% of ADA positive subjects). Analyses of TEAEs in selected SMQs (hypersensitivity, anaphylactic reaction, and angioedema) by ADA status revealed no clearly increased risk in ADA positive patients. These data need to be interpreted cautiously though, as the evaluated SMQs include several PTs, which frequently occur in ALS patients. Nevertheless, it is considered reassuring that of the patients with multiple tofersen related

pyrexia events, only two had positive ADA titers, whereas two patients had negative ADA status. No impact of plasma anti-tofersen antibodies on the incidence of the serious neurological ADRs (myelitis, radiculitis, papilloedema, or aseptic meningitis) was observed, which occurred in 4 (2.7%) patients with ADA positive status and in 6 (4.1%) with negative ADA status in Pool ABCL1. There appears to be no documented evidence that ADAs are produced intrathecally. Consequently, any ADAs measured in CSF would require either very high plasma ADA titres or disruption of the BBB. Additional measurement of ADAs in CSF would therefore be of questionable value regarding safety. The applicant did not identify an impact of ADAs in plasma on tofersen efficacy based on limited data. Since ASOs bind to their RNA substrate within cells and thus will not compete with circulating antibodies, neutralising ADA assays are not relevant for ASOs in simple saline formulation and have not been developed. Nevertheless, circulating ADAs have the potential to alter the distribution and clearance of tofersen, which could further impact effectiveness. However, the finding of a 32.2% decrease of tofersen plasma clearance in the presence of ADAs is somewhat questionable because of unlikely results. Due to the high prevalence of ADAs, an additional safety analysis of overall safety (and not only of safety topics of interest) by ADA status is therefore not anticipated to provide relevant new safety insights. The applicant plans to further evaluate the impact of ADAs on efficacy, on the incidence of serious neurological events and other areas of interest which may arise, in the ongoing clinical studies, which is endorsed.

In the provided subgroup analyses, it is striking, that in the overall tofersen 100 mg population (Pool ABCL1), CSF protein increased was reported as AE in 21/45 (46.7%) European vs. only 12/95 (12.6%) North American patients, and CSF WBC count increased was reported as AE in 12/45 (26.7%) European vs. 11/95 (11.6%) North American patients. Further evaluation indicates that this imbalance could be explained by differences in interpretation of CSF abnormalities, as the mean and median CSF WBC and protein values at baseline and across study visits were generally similar in European vs. North American patients. No clear differences in the risk of neuro-inflammatory SAEs by region were found. The provided subgroup analyses did not reveal any apparent differences in tofersen safety by age, sex, or ALS disease progression status, although subgroup analyses were generally limited due to the small subject numbers, and a higher risk of tofersen for more severe AEs (i.e. CTCAE grade ≥ 3 AEs, SAEs) in subjects with faster ALS progression cannot be excluded with absolute certainty. Also, data in older subjects is limited: The 'pivotal' study included only 9 tofersen and 5 placebo subjects ≥ 65 years, 20 subjects in the total tofersen 100 mg population were ≥ 65 years old, 2 of whom were ≥75-78 years; and 22 subjects ≥ 65 years were exposed with any dose of tofersen. Further evaluation of older tofersen treated patients by additional age categories is therefore not anticipated to provide meaningful results. As use of tofersen is also considered relevant in patients ≥ 65 years given the underlying disease, appropriate information on the data limitations in older patients has been included in SmPC section 4.2. No meaningful subgroup analyses by race or site of ALS onset could be provided.

No dedicated human interaction studies with tofersen have been performed. The applicant's statement in the SCS regarding absence of noticeable potentiation of common side effects of concomitant medications and absence of AEs suggestive of a potential drug-drug interaction is somewhat difficult to follow, as no details on these conclusions have been provided. Nevertheless, relevant interactions with concomitant medications used for sedation during the LP procedure would presumably occur with close temporal relationship of tofersen administration. In this context it is considered reassuring, that evaluation of AEs which occurred more frequently (≥ 5%) during the first 24 hours in the tofersen vs. placebo group of study 101 part C (pain in extremity, back pain, myalgia, and fatigue) does not raise clear concerns in this regard. 62% of the population of study 101 part C used concomitant riluzole, which is also associated with pain (common). Analyses of pain related AEs reported during study 101 part C did not clearly show a potentiation of pain related events in tofersen patients with concomitant riluzole.

A hypothetical potential for adverse effects attributable to SOD1 deficiency is derived from tofersen's mechanism of action. To date, findings in nonclinical and clinical studies of tofersen did not identify clear

risks attributable to SOD1 deficiency. However, a "toxic" threshold for reduced SOD1 protein and/or SOD1 enzymatic activity has not been identified and knowledge on SOD1 deficiency is sparse. Published case reports referenced by the applicant concern young children presenting with a neurodegenerative disorder involving progressive loss of motor function and spastic tetraplegia. Consequently, there is uncertainty, how SOD1 deficiency would present in (adult) ALS patients.

The updated data derived from the ongoing EAP (EAP; as of 28 Feb 2023) with an estimated number of up to 287 ALS patients treated with tofersen (of whom 67 may have been treated for one to two years) appear to be generally consistent with the safety profile derived from the clinical studies. In line with the serious ADRs of tofersen identified in the clinical studies, two related SAEs of myelitis (one leading to permanent tofersen withdrawal and resolved with sequelae [weakness], one ongoing as of 28 Feb 2023), one related SAE of radiculopathy (outcome unknown as of 28 Feb 2023), 2 related SAEs of aseptic meningitis, 2 related SAE of papilloedema and one related nonserious AE of lumbar radiculopathy (leading to permanent tofersen discontinuation, resolved within days) were reported. Among the AEs related to tofersen reported during the EAP, three events of "intercept product dispensing error" are listed. The applicant clarified that the product dispensing errors occurred at the warehouse level due to a failure of the internal labeling procedures but not due to any similarity in the name, packaging, or appearance of the drugs concerned tofersen and anifrolumab, respectively. Therefore, changes to the packaging or labelling of the marketed version of tofersen are not considered warranted at present. Apart from this no clear new safety signal arises from the EAP. One SAE (PT) of intervertebral discitis was considered possibly related to tofersen by the HCP. A causal relationship of the event of intervertebral discitis with tofersen cannot be fully excluded, although the inflammatory process did not concern the nervous system. However, based on the patient's confounding medical history, the course of the event (resolved without sequelae) as well as the fact, that this was the only event of discitis in the EAP or the CDP, the applicant's proposal not to include intervertebral discitis at the present time but to monitor for additional events can be agreed. The patient in whom an SAE of visual hallucination was reported one day after the first tofersen administration, experienced further episodes within 30 hours of subsequent doses. All episodes resolved within 24 hours, apparently decreased in severity with subsequent administrations, improved with anxiolytics and were rather considered suggestive of anxious manifestations and assessed as probably unrelated to tofersen by the HCP. A single event of hallucination was reported in the CDP (cutoff date 28 Feb 2023), however this event was considered as mild, unrelated, and did not occur within 24 hours of dosing. Therefore, labelling of hallucination is currently not considered warranted by the CHMP.

Additional safety data needed in the context of a MA under exceptional circumstances

The number of tofersen exposed subjects in the clinical studies (N=166) and, taking the difficulties of causality assessment in uncontrolled ALS studies into consideration, in particular the controlled exposure in the pivotal study (72 tofersen and 36 placebo patients) is rather limited.

The CHMP agreed on a data generation plan / SOBs so additional safety data could be generated during the post-authorisation phase (see also section 2.6.6 Discussion on clinical efficacy, Additional efficacy data needed in the context of MA under exceptional circumstances).

2.6.10. Conclusions on the clinical safety

Taken together, the safety profile of tofersen is considered acceptable.

The CHMP considers the following measures (SOB) necessary to address the missing safety data in the context of a MA under exceptional circumstances:

- 233AS102 An Extension Study to Assess the Long-Term Safety, Tolerability, Pharmacokinetics,

and Effect on Disease Progression of BIIB067 Administered to Previously Treated Adults with Amyotrophic Lateral Sclerosis Caused by Superoxide Dismutase 1 Mutation.

- 233AS401 An observational registry-based study to evaluate the long-term safety of tofersen in patients with SOD1-ALS.
- Submission of yearly updates on any new information concerning the safety and efficacy of tofersen

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 39: Summary of safety concerns

Important identified risks	• Myelitis and/or radiculitis • Increased intracranial pressure and/or papilloedema
Important potential risks	• None
Missing information	• None

2.7.2. Pharmacovigilance plan

Table 40: Summary of ongoing and planned additional pharmacovigilance activities

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
<u>Category 1</u> – Imposed mandatory additional pharmacovigilance activities that are conditions of the marketing authorisation				
• None	• None	• None	• None	• None
<u>Category 2</u> – Imposed mandatory additional pharmacovigilance activities that are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
233AS401 An observational registry-based study to evaluate the long-term safety of tofersen in patients with SOD1 ALS **<u>Status:</u>** Planned	*<u>Primary Objective:</u>* • Describe demographic and clinical characteristics of SOD1-ALS participants at baseline, and stratified by tofersen treatment status • Estimate the incidence of SAEs among all participants and stratified by tofersen treatment status, including serious neurologic events previously reported in clinical trial participants (e.g., myelitis, radiculitis, aseptic meningitis, increased intracranial pressure and/or papilloedema) *<u>Secondary Objectives</u>:* • Disease progression measured by the	• Myelitis and or radiculitis • Increased intracranial pressure and/or papilloedema	• Protocol submission • Interim progress report* • Continued evaluation	Within 6 months after the EC decision on this MAA 12 months after the Agency's approval of the final protocol and annually thereafter. With annual reassessment

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
	ALSFRS-R as rate of change over time in total scores • Occurrence of relevant clinical outcomes including permanent ventilation and feeding tube placement as well as changes in pulmonary function measured by FVC and SVC • Variant-specific survival • Reports of new comorbid conditions, pregnancy and pregnancy outcome, and hospitalisations (including reason for hospitalisation) not reported as SAEs • Treatment discontinuation			
233AS102 An Extension Study to Assess the Long-Term Safety, Tolerability, Pharmacokinetics, and Effect on Disease Progression of BIIB067 Administered to Previously Treated Adults with Amyotrophic Lateral Sclerosis Caused by Superoxide Dismutase 1 Mutation **Status:** Ongoing	*Primary Objective:* • Evaluate the long-term safety and tolerability of BIIB067 in participants with SOD1-ALS. *Secondary Objectives:* • Evaluate the PK, PD, biomarker effects, and efficacy of BIIB067 administered to participants with ALS and confirmed SOD1 mutation.	• Myelitis and or radiculitis • Increased intracranial pressure and/or papilloedema	• Final report	30 September 2025
Yearly updates on any new information concerning safety and efficacy of tofersen **Status:** Planned	In order to ensure adequate monitoring of safety and efficacy of tofersen in the treatment of patients with SOD1-ALS, the MAH shall provide yearly updates on any new information concerning the safety and efficacy of tofersen.	• Any new information concerning safety and efficacy of tofersen	Annual report	Annually (with annual reassessment)
Category 3 – **Required additional pharmacovigilance activities**				
• None	• None	• None	• None	• None

2.7.3. Risk minimisation measures

Table 41: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important identified risks		
Myelitis and/or radiculitis	***Routine risk minimisation measures:*** • SmPC Sections 4.4 and 4.8. • PIL Sections 2 and 4. • Legal status: Prescription only medicine. ***Additional risk minimisation measures:***	***Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:*** • DCT for collection of additional information relating to reported events of myelitis and/or radiculitis

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	• None	***Additional pharmacovigilance activities:*** • Study 233AS102 • Study 233AS401
Increased intracranial pressure and/or papilloedema	***Routine risk minimisation measures:*** • SmPC Sections 4.4 and 4.8. • PIL Sections 2 and 4. • Legal status: Prescription only medicine ***Additional risk minimisation measures:*** • None	***Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:*** • DCT for collection of additional information relating to reported events of papilloedema ***Additional pharmacovigilance activities:*** • Study 233AS102 • Study 233AS401
Important potential risks		
None	None	None
Missing information		
None	None	None

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.0 is acceptable.

The applicant is reminded that in case of a Positive Opinion, the body of the RMP and Annexes 4 and 6 (as applicable) will be published on the EMA website at the time of the EPAR publication, so considerations should be given on the retention/removal of Personal Data (PD) and identification of Commercially Confidential Information (CCI) in any updated RMP submitted throughout this procedure.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 25 April 2023

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request to omit certain particulars from the labelling as per Art.63.3 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

- Due to the limited space on the immediate label (vial of 20ml size), the QRD group accepted the omission of the full and allowed the use of minimum particulars applicable for small immediate packaging units.

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Qalsody (tofersen) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

ALS is a relentlessly progressive neurodegenerative disorder that causes muscle weakness, disability, and eventually death, with a median survival of three to five years.

Historically, ALS was identified as a clinical syndrome distinguishable from other motoneuron diseases such as primary lateral sclerosis, primary muscular atrophy, and progressive bulbar palsy, based upon the location of first symptom and the extent to which anterior horn cells or corticomotor neurons are initially involved. However, it is increasingly evident that ALS is clinically and pathophysiologically diverse.

Since 1993, mutations in more than forty genes are linked to ALS, the most frequent are those in the SOD1 gene encoding the essential antioxidant enzyme copper zinc superoxide dismutase. SOD1-ALS is a primarily autosomal-dominant disorder representing approximately 2% of the ALS population. The distribution of SOD1 mutations differs markedly even among apparently similar populations (i.e., Netherlands and Belgium, Ireland and England) and the same mutations in different populations can be associated with distinct clinical presentations. The clinical phenotype is highly variable and patients with

a particular SOD1 mutation show intrafamilial differences in the severity of symptoms and the speed of disease progression. The median survival time appears to be 2.3 years (Opie-Martin 2022).

3.1.2. Available therapies and unmet medical need

A treatment for SOD1-ALS has not been approved yet. Also, for ALS in general, the only treatment approved in Europe is riluzole, which was shown in 1996 (EU) to prolong survival based on 2 studies that demonstrated that the time to tracheostomy or death was prolonged for approximately 2 months in participants receiving riluzole compared to those receiving placebo.

The intended patient population for treatment with tofersen is SOD1-ALS.

3.1.3. Main clinical studies

The applicant has completed study 233AS101, consisting of phase 1/2 parts A and B and phase 3 part C. Part C of study 101 is intended to support the efficacy and safety of tofersen together with the OLE study 233AS102 (further referred to as study 102).

Study 101 Part C was a Phase 3, randomized, double-blind, placebo-controlled study comparing tofersen 100 mg administered by intrathecal bolus injection with placebo. A total of 108 participants with weakness attributable to ALS and a confirmed SOD1 mutation were randomized 2:1 to receive tofersen or placebo for approximately 28 weeks (3 loading doses followed by 5 maintenance doses).

Study 102 is an ongoing, multicenter, open-label, long-term extension study to assess the long-term safety, tolerability, PK, PD, and efficacy of tofersen administered by IT injection to participants with SOD1-ALS who completed Part C of Study 233AS101. Of the 159 eligible participants who completed Study 101, a total of 139 participants enrolled from Study 101 Parts A, B, and C: 44 participants enrolled from Study 101 Parts A and B and 95 participants enrolled from Part C.

3.2. Favourable effects

In study 101 Part C, a robust reduction in total CSF SOD1 protein was observed at Week 28 in the tofersen group compared to the placebo group (difference in geometric mean ratios for tofersen to placebo of 38% and nominal p <0.0001) in the mITT population. Also, in the ITT population, a reduction in total CSF SOD1 protein was observed at Week 28 in the tofersen group compared to the placebo group (difference in geometric mean ratios for tofersen to placebo of 34% and post-hoc nominal p < 0.0001).

Reduction in plasma NfL levels from baseline to Week 28 was observed in both populations (mITT and non-mITT), with a 67% reduction (difference in geometric mean ratios for tofersen to placebo) and nominal p < 0.0001 in the mITT population. In the ITT population this difference in geometric mean ratios for tofersen to placebo was 60% (*post hoc* nominal p < 0.0001). Robust reductions (60-70%) in plasma and CSF NfL from baseline were maintained up to week 104.

With respect to the primary analysis for the change in ALSFRS-R total score over 28 weeks as the primary endpoint, a statistically non-significant difference of 1.2 points was found in favour of tofersen in the mITT population using JRT in conjunction with MI for withdrawals other than death (p=0.9689). *Post hoc* analyses performed in the overall ITT population, adjusting for baseline NfL as a covariate rather than disease duration and including it also as part of the imputation model, showed a 2.1-point treatment difference in favor of tofersen with greater statistical differentiation, but without reaching significance (95% CI: -0.33, 4.54; JRT+MI nominal p = 0.5015; ANCOVA+MI nominal p = 0.0904).

Similar trends favouring tofersen treatment at week 28 were observed for SVC, HDD megascore and PROs such as ALSAQ-5, FSS, ED-5D-5L.

With respect to survival, time to death or PV and time to death were assessed as key secondary endpoints in Study 101 Part C. In the mITT population, a similar proportion of participants in the placebo (2 participants [9.5%] with events of permanent ventilation) and tofersen groups (4 participants [10.3%] – 1 death due to congestive heart failure and 3 events of permanent ventilation) experienced events of death or permanent ventilation. No events occurred in the non-mITT population.

The analysis of the pooled double-blind data from study Part C and the open-label data from study 102 LTE at week 52 and 104 showed consistent results. Numerical differences favoring early-start tofersen (as compared with delayed-start tofersen) were also observed by week 52 across clinical outcome measures. For function on ALSFRS-R there was a difference of 2.3 points (95% CI -1.26, 5.82, nominal p=0.21) not adjusted for NfL (but for disease duration as originally prespecified). Values adjusted for NfL using a post-hoc analysis were: ALSFRS-R: 3.5-point difference (95% CI 0.40, 6.69; nominal p = 0.0272).

Nominally statistically significant differences between early treatment tofersen and placebo/delayed treatment tofersen were also observed for SVC and HDD megascore to week 52 for the ITT population using a post-hoc analysis with baseline plasma NfL as a covariate. Results were for respiratory strength SVC: 9.2 percent-predicted difference (nominal p = 0.0159), for muscle strength HHD megascore: 0.28 difference (nominal p = 0.0186) and for PROs of disease severity and quality of life ALSAQ-5: -10.3 difference (nominal p = 0.0044); FSS: -3.8 difference (nominal p = 0.1493); EQ-5D-5L: 0.2 difference (nominal p < 0.0001).

The applicant presented an analysis (3rd interim) with an additional year of follow-up (28 February 2023 data cut, 104 weeks), comparing early start tofersen with delayed start. For the change from baseline in ALSFRS-R at week 104, the difference between groups was 3.7 (CI 95% -0.7, 8.2 and nominal p value of 0.1004), i.e. almost the same difference as observed after 52 weeks. A high percentage of tofersen-treated participants experienced sustained stabilisation or even improvement in function, which is clearly inconsistent with the natural history of the progressive disease.

Although the decline in ALSFRS-R is not always linear, it is estimated that people living with ALS decline by an average of approximately 1 point per month. Participants in Studies 101 Part C and 102 lost 9.5 and 13.2 points (adjusted mean on ANCOVA+MI) over 24 months in the tofersen early-start and placebo/delayed-start groups, respectively, which is not consistent with the natural course of the disease.

These data in the CL population (Study 101 Part C and Study 102) appear consistent with observations for the patients from the Study 101 Parts A and B, who continued in Study 102, received at least 1 dose of tofersen (N=40) (ABL Cohort) and for whom disease progression has essentially been stabilized for over 3 years of follow-up. These participants experienced a mean change from Study 102 baseline to Week 168 (~3.2 years) of -2.3 points on ALSFRS-R, -2.4 on percent-predicted SVC, and -0.1 on HHD megascore.

In the early-start tofersen group, 19.5% of participants experienced improvement on the ALSFRS-R and 29.3% had stabilisation or improvement over 104 weeks. In the case of the placebo/delayed start tofersen treatment group, 11.6% showed improvement and 22.7% had stabilisation or improvement over 104 weeks.

Under the Raw Data Pilot project, a new event analysis was defined as change from baseline in ALSFRS-R total score with a deterioration greater than 10 points (< -10), 21 days of permanent ventilation assistance or death at any time point up to Week 124 (~17 months). A hazard ratio for this event analysis was calculated 0.70 (0.375-1-.309) and when adjusted for log(baseline NfL), the HR was 0.46 (0.241-0.883). The HR calculated by the applicant for time to death or PV was 0.47 and for time to

death, PV, or withdrawal due to disease progression HR was 0.50. These analyses indicated a better outcome for the patients with early tofersen treatment, especially when baseline NfL is taken into account, indicating an association of NfL and detrimental effect in patients' condition.

Survival was numerically in favour for early start tofersen. An extended duration of follow-up from baseline to 3.4 years showed that the majority of patients remain alive on-study, since the number of death-equivalent events were limited. The range of follow-up from 2.2 to 3.9 years exceeds the 2.3 years median survival for SOD1-ALS characterized in the literature. The survival benefit in the early-start participants, relative to the delayed-start participants, is unlikely to be attributed to enrolment of individuals with more slowly progressing disease.

A5V variant carriers, albeit being well studied and well characterised, are known to be associated with a median disease duration of approximately 1 to 1.2 years [Bali 2017; Opie-Martin 2022]. From a small subset of the participants in study 101 Part C and 102 who are A5V carriers (n=17), the median duration from ALS symptom onset to death or censoring was 1.9 years (range: 0.9 to 4.8 years) in the early-start group and 1.3 years (range: 0.7 to 2.9) in the delayed-start group, with 3 A5V carriers ongoing as of the data cut off. This is not in line with the usual disease progression course of A5V carriers and indicates a potential tofersen treatment benefit, but uncertainties due to small sample size and limitations of comparisons against historical controls should be also taken into account.

Cumulative distribution function analysis of ALSFRS-R changes from baseline at Weeks 52 and 104 reflects the probability of worsening defined by change from baseline in ALSFRS-R being less than the cut-off value, taking into consideration treatment discontinuation due to death, progressive disease, AE, and other subject withdrawal as the worst outcome. For a cutoff of -12, the rates of worsening in ALSFRS-R are approximately 32% and 44% for the early-start tofersen group and the placebo/delayed-start tofersen group, respectively. These show that participants in the placebo/delayed-start group tend to have a higher rate of worsening in ALSFRS-R at Week 52. Overall, the CDF analysis for ALSFRS-R supports the tofersen treatment effect with regards to a higher responder proportion at Weeks 52 and 104. However, the *post hoc* and descriptive nature of the analysis (including not showing uncertainty such as confidence intervals) need to be taken into account.

A consistency in the clinical measures results with trends in favour of tofersen treatment over time, over 104 weeks, is clearly observed. Even if some of the analyses rely heavily on strong assumptions, the results are not contradictory to the tofersen treatment effect.

The results from the Part A/B participants (ABL) should be interpreted with caution due to the significant washout periods, variable dosing history, and the more slowly progressing nature of their disease. However, even in slower-progressing *SOD1*-ALS patients, the disease is associated with progressive declines in strength and function. These participants have experienced an apparent stabilisation of function and strength over more than 3 years of follow-up, with a reduction of only 2.3 in ALSFRS-R total score over 168 weeks (approximately 39 months).

The applicant has submitted a supportive data generation plan and specific obligations for a MAUEC, which includes the conclusion of studies 102 LTE and 303 (ATLAS), registry-based study 233S401 and collaboration with ALS-NHC (primarily US-based) and TRICALS /European sites). The applicant has provided all information on study 303 available up to 26 June 2023. Study 303 is well in progress with over 68% of the planned presymptomatic at-risk carriers of SOD1 mutation already enrolled.

3.3. Uncertainties and limitations about favourable effects

No statistically significant difference between tofersen and placebo groups was observed on the primary analysis of ALSFRS-R change from baseline to Week 28 in the primary analysis (mITT) population. Hence, study 101 Part C failed to provide confirmatory evidence of efficacy in SOD1-ALS.

There is large heterogeneity with several variants with very diverse characteristics in a very small proportion (~2%) of ALS patients with the SOD1 mutation. However, baseline characteristics regarding variants are not considered to have favoured the tofersen group.

It is acknowledged that the prognostic and predictive value of NfL in ALS is not fully established yet and NfL cannot be considered as a validated surrogate endpoint for predicting efficacy. However, NfL is being used in most of the clinical trials in ALS and it is currently recognised as an important and reliable biomarker for neurodegeneration in ALS (Thompson et al 2022, Brousse et al 2023, Jiang et al 2022, Mead et al 2023, McCluskey et al 2023 and Sanchez-Tejerina et al 2023). The clinical relevance of treatment-driven reductions in neurofilament is supported by a significant body of literature. NfL may not only be a prognostic marker in ALS but also a marker for prediction of clinical response. The majority of the SAG-N experts considered that NFL levels can be used for predicting a clinical effect in patients with ALS at least on a cohort level. There is uncertainty in this prediction mainly due to the timelines - time since the NFL change to the occurrence of the clinical effect - and the potential of prediction of a clinically relevant effect because there is no clear definition on what constitutes a clinically relevant effect in ALS (e.g., a threshold of change in a clinical scale) besides a change in survival which is considered as clinically relevant by all SAG-N experts.

NfL cannot be considered as a validated surrogate endpoint for predicting efficacy in ALS for the time being. However, there is a strong biological plausibility (supported by nonclinical data), that the lowering of toxic SOD1 exerts disease-modifying effects of tofersen, which can be pharmacodynamically measured by an appropriate neurodegeneration biomarker, NfL. A number of literature publications consistently show that neurofilament levels are prognostic for disease progression and survival in ALS. These reductions in SOD1 protein and consequently NfL could not be translated immediately into clear differences in ALSFRS-R and other clinical outcome measures, indicating a potential delay in the treatment effect. Similar observations have been made in cases with other ASOs (milasen and jacifusen). It should be pointed out that baseline plasma NfL levels were approximately 23% higher in the early-start group than in the placebo/delayed-start group. This suggests that the early-start tofersen group was at a higher risk for faster disease progression and shorter survival compared to the placebo/delayed-start tofersen treatment group. These higher plasma NfL levels in the early start group were not in favour of tofersen treatment.

After the analysis FDA performed with the difference between groups in the change from baseline to week 28 in ALSFRS-R and plasma NfL levels, a correlations exploration was performed in the EMA Raw Data Pilot Project. In this exploration weak correlations were observed between the change from baseline to week 28 in ALSFRS-R and plasma NfL levels at certain tome points. Clarifications for the correlations between NfL and clinical function and potential discrepancies have been provided. The relationship between the reduction in NfL and clinical function cannot be adequately assessed using correlation coefficients without adjustment for baseline NfL, as this approach does not take into account variable natural disease progression across participants. It is acknowledged that the partial correlations presented by the applicant are in line with the hypothesis of a relationship between changes in NfL and clinical outcomes.

The observed effects in important secondary endpoints in study 101 Part C were statistically non-significant, but showed consistency over a period of time at weeks 28, 52 and 104.

With respect to survival the numbers are too low for the duration of the placebo-controlled study 101 Part C (28 weeks) to allow any firm conclusions.

When combining double-blind data from study 101 Part C and uncontrolled data from study 102 LTE, a nominally statistically significant difference of 3.5 (p=0.0272) in the change of ALSFRS-R from baseline to week 52 between early and placebo/delayed start tofersen group was observed with a post-hoc analysis after baseline NfL adjustment. However, there is considerable uncertainty on the size of the potential treatment effect, which cannot be reliably quantified based on the available controlled and uncontrolled data.

At week 104 there were 5 (13.9%) deaths in the placebo/delayed start tofersen group and 8 (11.1%) in the early start tofersen group, leading to comparable percentages. The applicant has performed analyses for three different time to event endpoints: Time to death or PV, Time to death, Time to death or PV or withdrawal due to disease progression and calculated the respective Hazard ratios. For time to event endpoints with events 'death or PV', 'death' and 'death, PV or withdrawal due to disease progression', Cox regression including NfL as covariate showed HRs of 0.47 (95% CI (0.20,1.11)), 0.36 (95% CI (0.13,1.02)) and 0.50 (95% CI (0.25,0.99)), respectively, at the latest data cut-off. However, the overall number of events (deaths or PV) at the latest data cut-off (28 February 2023) is small, despite that strong assumptions were made for analysis.

It is acknowledged that comparisons to natural history or the natural course of the disease are difficult to interpret because this requires that natural history is characterized with high certainty, which may not be the case in this heterogeneous population.

3.4. Unfavourable effects

Immunostimulatory and proinflammatory effects have been associated with ASOs in multiple preclinical species and in humans. In the tofersen CDP, serious neuroinflammatory adverse reactions were identified with no occurrence in the placebo group of study 101 Part C. The incidence was higher than would have been expected for the study population and the biological plausibility. Some patients experienced more than one type of neuroinflammatory ADR. Apart from papilloedema, the identified neuroinflammatory events correlated with non-clinical findings. All these events were associated with increased CSF white blood cells and CSF protein of varying degrees and included:

Myelitis and radiculitis

Myelitis (PTs myelitis, myelitis transverse, and neurosarcoidosis) and radiculitis (PTs radiculopathy and lumbar radiculopathy) occurred in 6 participants. All events of myelitis were SAEs. No pattern with regard to number of tofersen doses received before the onset of these SAEs was found (range after 1st to 24th dose). Two subjects with ADRs of myelitis received immunomodulatory therapy, however, one subject had a history of neurosarcoidosis. Two events of myelitis were asymptomatic and diagnosed based on imaging and or laboratory findings. Overall, five patients with SAEs of myelitis or radiculitis had inflammatory findings in the spinal MRI. In two of these patients, tofersen was administered while inflammatory findings were present, in two subjects, tofersen was discontinued or only resumed after imaging was improved, respectively, in one subject no information was available in this regard. In two of four SAEs of myelitis and in both SAEs of radiculopathy, tofersen was maintained. Associated symptoms of all SAES of myelitis/radiculitis resolved within days to months, although in one subject the SAE of radiculopathy was considered resolved with sequelae of bilateral foot sensory loss after a long period (9 months).

Papilloedema

In pool ABCL1 (as well as in the total CDP) 4/147 subjects reported SAEs related to papilloedema (PTs papilloedema, and intracranial pressure increased); in addition, non-serious events of papilloedema, ICP increased and CSF pressure increased were reported with an overall incidence of papilloedema of 8/147 subjects (5.4%). All four SAEs were symptomatic with headache in all and visual disturbances in three subjects concerned, and all four SAEs demonstrated papilloedema as a feature, which however was not always present at the beginning of the event. One SAE of papilloedema/increased ICP resolved with sequelae of improved but ongoing symptoms, which led to permanent tofersen discontinuation (after tofersen interruption and subsequent bimonthly dosing). One SAE was not resolved as of 28 Feb 2023 with unchanged tofersen treatment; however, the clinical symptoms and papillary oedema had disappeared. Two SAEs resolved without sequelae after approx. 7 and 8 months, respectively, in one case no action was taken with tofersen, in the other, treatment with tofersen was interrupted. Treatment of SAEs of papilloedema/ICP increased included amongst others long-term treatment with acetazolamide in several subjects, and IV methylprednisolone with tofersen dosing visits in one subject.

Aseptic meningitis

In the CDP, 6 patients experienced aseptic meningitis (PTs meningitis chemical or aseptic meningitis), these events were serious in 2 and non-serious in 4 subjects. Both SAEs of aseptic meningitis were associated with typical symptoms and negative microbial studies. Both SAEs resolved, after 10 days in a subject, in whom study treatment was permanently discontinued, and after less than 4 weeks in a subject, in whom study treatment was maintained.

In tofersen treated patients, a mean increase in CSF leukocytes and CSF protein was found, which appeared to stabilize each after approx. 500 days of treatment with continued tofersen exposure at above the four-fold of the upper normal value for leukocytes (i.e. at above 20 leukocytes x 106/L after approx. 425 days) and at nearly twice the upper normal value for protein (i.e. at approx. 1000 mg protein/L after approx. 500 days). In the total tofersen 100 mg population (Pool ABCL1), 26.5% subjects each reported AEs of CSF WBC increased or CSF protein increased, respectively. Per se these were not treatment limiting.

Apart from CSF abnormalities, the most common adverse drug reactions are pain (including the PTs pain, back pain, and pain in extremity; reported in 66.0% subjects in the total tofersen pool ABCL1), fatigue (28.6%), arthralgia (34.0%), and myalgia (19.0%), respectively, which were all identified based on a > 5% higher incidence in tofersen vs. placebo subjects in study 101 part C. Analyses of TEAEs reported ≥ 5% more frequently within 24 hours of dosing in the tofersen vs. placebo group of study 101 part C indicate, that the identified ADRs of pain in extremity, back pain, myalgia, and fatigue, respectively frequently occur within 24 hours of tofersen administration.

In addition, pyrexia was identified as an adverse reaction due to positive rechallenge experiences in participants receiving tofersen and as pyrexia occurred in 9.1% subjects within 24 hours of tofersen administration in Pool ABCL1. Pyrexia was reported in 18.4% subjects in the total tofersen 100 mg population (Pool ABCL1), overall, no pyrexia event was severe or serious, and no increase in the severity of pyrexia with longer treatment duration was recognizable. In all of the patients with a positive pyrexia rechallenge no action was taken with study treatment as a result of the event and no precautionary measures for tofersen related pyrexia (e.g., antipyretics) were taken around the time of any pyrexia events. Of the patients with multiple tofersen related pyrexia events, only two had positive ADA titers, whereas two patients had a negative ADA status. Pyrexia is an ADR that is shared by multiple ASO drugs and may be mediated by an immune response to the ASO.

Lumbar puncture (LP) procedures are associated with known risks (e.g. procedural pain, post-lumbar puncture syndrome, and headache) and 80.6% of subjects in either treatment group of study 101 part C experienced LP related events. During the clinical study program, one LP related, non-serious event of propionibacterium infection of the CSF was reported, that was of moderate severity and required intravenous antibiotic treatment for two weeks. Apart from this, no events suggestive of a serious CNS infection and no SAE of (CNS) infection due to the LP was reported in the CDP. SmPC Section 4.4 was amended to warn about the risk of CNS infection (amongst other established AEs of an LP procedure) and lumbar puncture related adverse reactions have been adequately implemented in section 4.8 of the SmPC.

As of 15 Jul 2022, 63.3% of the total tofersen 100 mg safety population (pool ABCL1) developed positive ADAs in plasma, the majority of which remained persistent (in 81.7% of ADA positive subjects). Serious neurological ADRs (myelitis, radiculitis, papilloedema, or aseptic meningitis), occurred in 4 (2.7%) patients with ADA positive status and in 6 (4.1%) with negative ADA status (Pool ABCL1).

As of the updated cut-off date of 28 Feb 2023, an estimated number of 287 patients have been treated with tofersen during an ongoing EAP, 67 of whom may have received tofersen for at least one year. The data derived from the EAP appear to be generally consistent with the safety profile derived from the clinical studies. Among the AEs related to tofersen reported during the EAP, three events concerned "intercept product dispensing error". However, the applicant clarified, that the product dispensing errors occurred at the warehouse level due to a failure of the internal labelling procedures but not due to any similarity in the name, packaging, or appearance of the drugs concerned tofersen and anifrolumab, respectively. Therefore, changes to the packaging or labelling of the marketed version of tofersen are not considered warranted at present.

3.5. Uncertainties and limitations about unfavourable effects

The safety profile of tofersen is based on a limited number of exposed patients (147 SOD1-ALS patients have received at least 1 dose of 100 mg tofersen, the intended and highest evaluated dose), and on a limited controlled exposure in study 101 part C considered pivotal for this application (i.e. 72 tofersen and 36 placebo subjects treated for 24 weeks). However, with the updated safety data as of 28 Feb 2023, long-term treatment is nevertheless available from 116 (95) patients having received 100 mg tofersen for at least one (two) year(s) with a maximum treatment duration of 245 weeks.

A clear risk of tofersen for hypersensitivity reactions cannot be concluded at present. The applicant will continue to monitor for hypersensitivity reactions in the ongoing tofersen trial, EAP and in the post-marketing setting.

The significance of an imbalance found with regard to increase in QTc >30 to 60 ms in tofersen vs. placebo subjects (11.3% vs. 5.6%) in study 101 part C is unclear. There was no increase in relative to placebo control in the incidence of cardiac AEs associated with delayed ventricular repolarisation in tofersen treated patients. In routine ECG monitoring, an increase in QTc > 60 ms, or QTc values > 480 ms or > 500 ms was not reported in this study part and was each reported. Also, in pool ABCL1 (as of 28 Feb 2023), AEs reported in the cardiac disorders SOC and in the investigations SOC related to ECG abnormalities, respectively were generally infrequent (<3% by PT, apart from tachycardia reported in 3.4%) and were all considered unrelated to treatment. A hERG assay as well as the provided PK/PD modelling of QTc data from the clinical studies was not indicative of a proarrhythmic potential of tofersen.

Analyses of TEAEs in selected SMQs (hypersensitivity, anaphylactic reaction, and angioedema) by ADA status revealed no clearly increased risk of these events in ADA positive patients. However, these data are limited as there is overlap between the PTs of the evaluated SMQs and frequent ALS symptoms. Limited data (including PK/PD modelling of SOD1 protein and NfL) also did not identify an impact of ADAs

in plasma on tofersen efficacy. The applicant plans to further evaluate the impact of ADAs on efficacy as well as on the incidence of serious neurological events and other areas of interest, which may arise in the ongoing clinical studies, which is endorsed. The provided subgroup analyses did not reveal any apparent differences in tofersen safety by age, sex, or ALS disease progression status, although subgroup analyses were generally limited due to the low subject numbers, and a higher risk of tofersen for more severe AEs (i.e. CTCAE grade ≥ 3 AEs, SAEs) with tofersen in subjects with faster ALS progression cannot be excluded with absolute certainty. A higher reporting rate of AEs of CSF protein increased in European vs. North American patients (46.7% vs. 12.6%) and also of CSF WBC count increased (26.7% vs. 11.6%) could be explained by differences in interpretation of CSF abnormalities, as the mean and median CSF WBC and protein values at baseline and across study visits were generally similar in European vs. North American patients. No clear differences in the risk of neuro-inflammatory SAEs by region were found.

A causal relationship of one event of intervertebral discitis, that was reported during the Expanded Access Program, with tofersen cannot be fully excluded, although the inflammatory process did not concern the nervous system. However, the patient had a confounding medical history, the event resolved without sequelae and based on the fact, that this was the only event of discitis in the EAP or the CDP, intervertebral discitis is not included to SmPC section 4.8 at the present time and the applicant will monitor for additional events.

The available data for tofersen do not indicate a clear risk of thrombocytopenia, coagulation abnormality or bleeding, or renal toxicity. However, thrombocytopenia, coagulation abnormalities as well as renal toxicity have been observed after administration of some subcutaneously and intravenously administered ASOs. Based on class effects data, these risks cannot be excluded for tofersen and should be further followed. A respective warning has been added to SmPC section 4.4.

Hydrocephalus was not reported during the tofersen CDP so far. However, hydrocephalus has been reported with intrathecally administered ASOs (nusinersen and tominersen) and the risk of hydrocephalus should be further followed.

3.6. Effects Table

Table 42: Effects table for Qalsody; treatment of adults with amyotrophic lateral sclerosis (ALS), associated with a mutation in the superoxide dismutase 1 (SOD1) gene] (data cut-off:28 Feb 2023).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Predefined ALSFRS-R change to week 28	ALSFRS-R change from baseline to week 28 for the predefined mITT (faster progressing subgroup)	point	Tofersen (n=72) -6.98	Placebo (n=36) -8.14	UnCert: difference **1.2** (CI -3.2, 5.5), p-value (Joint rank+MI) **0.9689**	Study 101 Part C
ALSFRS-R change to week 28 *Post hoc*	ALSFRS-R change from baseline to week 28 - Post hoc analyses with baseline plasma NfL as a covariate (from Study 101 Part C Baseline - ITT population)	point	Tofersen (n=72) -4.10	Placebo (n=36) -6.20	UnCert: difference **2.1** (CI -0.3, 4.5), Nominal p-value (ANCOVA + MI) **0.0904**	Study 101 Part C

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	Referenc es
ALSFRS-R change to week 52 *Post hoc*	ALSFRS-R change from baseline to week 52 - Post hoc analyses with baseline plasma NfL as a covariate (from Study 101 Part C baseline - ITT population)	Point	Tofersen early start (n=72) -6.00	Placebo/D elayed start tofersen (n=36) -9.50	**SoE:** difference **3.5** (95% CI 0.4, 6.7), p-value (ANCOVA+MI) **0.0272** **UnCert:** pooling of double blind and uncontrolled data	Studies 101 Part C and 102 (ISE SAP V3.0)
ALSFRS-R change to week 104 *Post hoc*	ALSFRS-R change from baseline to week 52 - Post hoc analyses with baseline plasma NfL as a covariate (from Study 101 Part C baseline - ITT population)	Point	-9.5	-13.2	**SoE:** Adjusted mean difference (95% CI): **3.7** (-0.7, 8.2), ANCOVA+MI: p=0.1004, Original JRT: p=0.0835, Alternative JRT: p=0.0335	Responses to Day 120 LoQ
Responder-like analyses	Cumulative probability of failure at week 52§		Tofersen early start (n=72) 35%	Placebo/D elayed start tofersen (n=36) 47%	**SoE:** cumulative probability of failure for a cut-off of -10 in ALSFRS-R, PV or death, a rate of about 35% and 47% for the failure rates corresponds to early start and placebo delayed start tofersen treatment group, respectively.	Raw Data Pilot Project
Unfavourable Effects						
			Tofersen 100 mg	Placebo		
Myelitis	Incidence	(%)	ABCL1: 2.7	not reported in placebo group of study 101 part C (pool RC2)	Higher incidence than expected; always associated with CSF abnormalities of varying degrees; mononuclear cell infiltrates in the spinal cord and nerve roots in toxicology studies	(1), (2)
Radiculitis	Incidence	(%)	ABCL1: 2.7	Not reported in pool RC2	(see myelitis)	(1), (2)
Papilloedem a/ intracranial pressure increased	Incidence	(%)	ABCL1: 5.4*	Not reported in pool RC2	Higher incidence than expected reported in tofersen patients only; always associated with CSF abnormalities of varying degrees; postulated to be caused by CNS inflammation; no corresponding pre-clinical findings;	(1), (3)
Aseptic meningitis	Incidence	(%)	ABCL1: 4.1	No reported in pool RC2	Associated with several intrathecally administered Active substances; (high incidence of CSF WBC/protein increased with tofersen)	(1), (3)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
CSF WBC increased	Incidence of AE Any shift to high	(%)	ABCL1: 26.5 ABCL1: 91.2		Gradual rise in mean CSF WBC (to ~ 4xULN; stabilisation after ~ 500 days)**; increase in CSF WBC in 9-months non-human primate studies;	(1), (3)
CSF protein increased	Incidence of AE Any shift to high	(%)	RC1: 8.3 ABCL1: 26.5 ABCL1: 80.3	RC2: 2.8	Gradual rise in mean CSF protein (to ~ 2xULN; stabilisation after ~ 500 days)**; increase in CSF protein in 9-months non-human primate studies;	(1), (3)
Pain (including PT pain, back pain, pain in extremity)	Incidence	(%)	RC1: 41.7	RC2: 22.2	>5% higher incidence vs. pbo (also for associated PTs each);	(1)
Fatigue	Incidence	(%)	RC1: 16.7	RC2: 5.6	>5% higher incidence vs. pbo;	(1)
Pyrexia	Incidence	(%)	RC1: 4.2 Pool ABCL1: 18.4	RC2: 2.8	Pyrexia within 24 hrs of dosing in 9.1% ABCL1 patients; Positive rechallenge in multiple patients;	(1), (3)
Arthralgia/Myalgia/ Neuralgia	Incidence	(%)	RC1: 13.9 RC1: 13.9	RC2: 5.6 RC2: 5.6	>5% higher incidence vs. pbo;	(1)
Immuno-Genicity	Incidence in plasma - ADAs - persistent ADAs	%	ABCL1: 63.3 51.7		No apparent increase in events identified by SMQs (hypersensitivity, anaphylactic reaction, and angioedema) in ADA positive group; No apparent higher risk of serious neuroinflammatory ADRs in ADA positive group;	ISI, (2)
Risk related to the LP					Known risk of adverse reactions to LP procedures (e.g. headache, procedural pain, post-lumbar puncture syndrome); One propinoibacterium infection of CSF (moderate, non-serious, requiring antibiotic treatment) Risk of serious infection? (bacterial meningitis is known, very infrequent complication of LP)	
ASO class related risks					Thrombocytopenia and coagulation abnormalities, including acute severe thrombocytopenia, have been observed after administration of other subcutaneously or intravenously administered antisense oligonucleotides. Renal toxicity has been observed after administration of other subcutaneously and intravenously administered antisense oligonucleotides.	
Risk of Hydrocephalus					Reported with intrathecally administered ASOs, i.e. tominersen (secondary to aseptic meningitis), nusinersen	

Abbreviations: ASO – antisense oligonucleotide, ADA – antidrug antibody, CSF – cerebrospinal fluid, ICP – intracranial pressure; pbo – placebo; ISI – integrates summary of immunogenicity; LP – lumbar puncture; WBC – white blood cell;

Notes: (1) Study 101 part C evaluating tofersen 100 mg (Pool RC1) and placebo group (Pool RC2);
(2) Total tofersen 100 mg population from study 101 and 102 (Pool ABCL1, cut-off date: 15 Jul 2022).
(3) Total tofersen 100 mg population from study 101 and 102 (Pool ABCL1, cut-off date: 28 Feb 2023).
*including one patient with "CSF pressure increased"
** as of 15 Jul 2022
§ cumulative distribution functions of the change from baseline in the ALSFRS-R total score were generated for both treatment groups indicating the probability of failure (y axis) projected versus change from baseline as cut-off value defining failure, taking into consideration treatment discontinuation due to death, progressive disease, AE and other subject withdrawal as failure expressed as a decrease from baseline of -40 being lower than any other change from baseline.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The applicant has developed an ASO, tofersen, to target the increase and accumulation of toxic SOD1 in ALS. The mechanism of action is plausible and relevant and sustained reductions in SOD1 in CSF could be demonstrated with IT administration of tofersen. Indications of subsequent slowing of axonal injury and neurodegeneration derive from non-clinical and clinical pharmacology data by clear reductions in the neurodegeneration biomarker NfL. NfL is being increasingly acknowledged as an important biomarker for neurodegeneration in ALS.

However, the pivotal trial 101 Part C which used appropriate scales, did not confirm efficacy of tofersen in patients with SOD1 ALS. The observed effects were small and neither statistically significant, nor clinically relevant. Assay sensitivity was low probably for several reasons including study design with a too short placebo-controlled phase of only 28 weeks, suboptimal selection of variables for enrichment purposes, and large heterogeneity of the SOD1 ALS population.

However, consistent trends in favour of tofersen compared to placebo were observed at week 28. Furthermore, longer term data comparing early vs delayed start tofersen suggest a treatment effect of tofersen. These favourable trends continued up to week 104 in the CL-ITT group. Numerical differences consistently in favour of tofersen across several assessment scales in the context of sustained SOD1 and NfL decreases should be taken into consideration for a potential benefit in SOD1-ALS, which is an ultra-rare disease with a high unmet medical need. The data suggest that these reductions in SOD1 protein and consequently NfL result in a delayed effect on function as measured by ALSFRS-R and other clinical outcome measures, which is considered biologically plausible. The majority of the SAG-N experts considered that NFL levels can be used for predicting a clinical effect in patients with ALS at least on a cohort level. However, NfL is not an established biomarker with a predictive value in ALS. Explanations for the lack of strong correlations between changes in NfL and ALSFRS-R across the pairs of timepoints considered in the Raw Data Pilot Project were provided and these are acceptable.

Survival data are too limited to allow robust conclusions.

The main methodological concerns on tofersen efficacy are the lack of confirmatory evidence at week 28 and the difficulty to interpret *post hoc* analyses of pooled data across study 101 and its OLE study 102.

However, in agreement with the applicant's argumentation, comparison of the early-start and placebo/delayed-start groups can be considered reliable. The reasons are: 1) Study 101 Part C treatment assignments remain blinded such that any bias introduced by the open-label nature of the study would apply equally to both arms and 2) baseline disease characteristics were generally balanced between these groups by way of SOD1 variant type, use of riluzole and/or edaravone, and characteristics reflective of the stage of disease (time from symptom onset, total ALSFRS-R score, and percent-predicted SVC). Therefore, any survival benefit in the early-start participants relative to the delayed-start participants is unlikely to be attributed to enrolment of individuals with more slowly progressing disease.

"Responder-like" analysis with a cumulative probability of response also suggest that tofersen may have a disease modifying effect.

Although efficacy of tofersen in SOD1 ALS has not been proven in a confirmatory sense and that methodological uncertainties related to interpretation of pooled *post hoc* analyses remain, the clinical results support that there is a favourable trend of effect of tofersen treatment, which eventually could lead to at least a slowing or a stabilisation of the disease and, in some cases, even to an improvement in function. An additional, adequately powered, placebo-controlled study in SOD1-ALS is not considered operationally feasible, given the ultra-rare nature of the disease (see also discussion on clinical efficacy).

The main safety concern of tofersen relates to the serious neuroinflammatory ADRs of myelitis and radiculitis, papilloedema/increased intracranial pressure and aseptic meningitis, respectively, which were commonly reported each and all appear to involve the central nervous system. These ADRs were predominantly associated with typical symptoms of the respective type of adverse reaction but were sometimes asymptomatic and led to study treatment discontinuation or treatment interruption in some cases. Treatment applied for myelitis involved (amongst others) immunomodulatory treatment in single patients, treatment of SAEs of papilloedema/ICP increased included long-term treatment with acetazolamide in several subjects (which is regularly used for the treatment of papilloedema in clinical practice but not approved), as well as IV methylprednisolone with tofersen dosing visits in one subject. Treatment of aseptic meningitis involved symptomatic NSAIDS and steroids. Associated symptoms of SAEs of myelitis/radiculitis all resolved within days to months, although one SAE of radiculopathy resolved with sequelae (bilateral foot sensory loss) after 9 months. As of 28 Feb 2023, one of the 4 SAEs relating to papilloedema was reported ongoing, while accompanying symptoms appeared mainly resolved. Both SAEs of aseptic meningitis resolved.

The serious neuroinflammatory ADRs are considered manageable with standard of care diagnostics and treatment as proposed by the applicant. As there was high variability in manifestations, courses and required action in these SAE cases, and as no discernible risk factors for development of these ADRs were identified, the applicant's position not to make more specific recommendations in the SmPC can in general be agreed. The consequences regarding tofersen treatment (i.e., treatment discontinuation, interruption or continuation) in case of neuro-inflammatory adverse reactions should be decided upon by the treating physician at the individual patient level. All four patients with SAEs of papilloedema/increased ICP had typical symptoms such as headache or visual disturbance, which are clinically monitorable. Therefore, routine measurement of the opening pressure is not generally recommended with tofersen treatment. Nevertheless, an evaluation of the fundus of the eye before any LP is considered standard clinical practice.

Myelitis and/or radiculitis and papilloedema are included in the RMP as important identified risks, which is agreed as these are clinically serious events with potentially serious outcomes. Symptoms of myelitis/radiculitis can imitate or worsen typical ALS motor symptoms but also include sensory disorders and pain. Papilloedema is associated with increased intracranial pressure and usually presents with headache and visual disturbance, which can lead to vision loss, if untreated. In line with nusinersen, for which aseptic meningitis is also an identified risk, the applicant considers the risk of aseptic meningitis as not important, mainly because drug-induced meningitis is generally monitorable, transient, and does not lead to permanent sequelae, which could be agreed. Nevertheless, hydrocephalus has been reported with nusinersen and a case of hydrocephalus secondary to a sterile meningitis induced by the intrathecally administered ASO tominersen has been reported in literature. Although no events of hydrocephalus were reported in the CDP so far, this risk cannot be excluded for tofersen and should be followed further.

In addition, the safety profile of tofersen is mainly characterised by very common events of pain (reported in 41.7% tofersen vs. 22.2% placebo patients), fatigue (16.7% vs. 5.6%), arthralgia, myalgia, and pyrexia.

Adverse events known to be frequently associated with lumbar puncture (LP) procedures like headache, procedural pain, and post-lumbar puncture syndrome also occur with tofersen treatment due to the mode of administration. A burden is certainly imposed on the patients during treatment with tofersen due to the repeated LP procedures (with bi-weekly intervals between the three loading doses and monthly intervals thereafter), however this was not quantifiable in the clinical studies, as LPs were also administered to placebo subjects.

Based on class effects data, the risks of thrombocytopenia, coagulation abnormalities and of renal toxicity cannot be excluded for tofersen and should be further followed.

3.7.2. Balance of benefits and risks

A mechanistic basis for the potential treatment effect of tofersen exists and has been described in sufficient detail. There is target engagement and a strong biological plausibility that the lowering of toxic SOD1 exerts a disease-modifying effect of tofersen, which can be pharmacodynamically measured by an appropriate neurodegeneration biomarker, increasingly used in ALS, i.e. NfL. However, at week 28 (placebo-controlled data) there was no statistically significant difference in the change from baseline on the ALSFRS-R between tofersen and placebo regardless of the analyses conducted. However, follow-up data up to at least week 104 showed results for all endpoints consistently favouring tofersen. Survival data and analyses with a cumulative probability of response also suggest that tofersen has the potential to modify the clinical course of SOD1 ALS.

Risks associated with tofersen treatment are not negligible but considered manageable.

The benefit/risk balance of tofersen for the treatment of patients with SOD1-ALS, a serious, relentlessly progressive and invariably fatal disease, is considered favourable based on the totality of evidence provided.

The CHMP did not consider the available data to be comprehensive to support a full MA as initially requested by the applicant but considered a MAUEC to be the most appropriate approach because of the rarity of the disorder and the improbability that comprehensive data can be generated.

The SOBs suggested by the applicant were accepted. In addition to the open-label long-term extension of Study 102 and ATLAS, the applicant confirmed their indefinite collaboration with two disease registries (ALS/MND-NHC and Precision ALS) and proposed an observational registry-based Study 233AS401 to evaluate the long-term safety of tofersen in patients with SOD1-ALS. The applicant has already provided a draft synopsis of the proposed Study 233AS401, which will also follow patients' disease progression measured by the ALSFRS-R as rate of change over time in total scores. Occurrence of relevant clinical outcomes, including permanent ventilation and feeding tube placement as well as changes in pulmonary function measured by FVC and SVC will also be measured.

3.7.3. Additional considerations on the benefit-risk balance

Patient representatives

The contribution of patient representatives was acknowledged and welcomed by CHMP. The points raised by one person receiving tofersen with "fluctuating effects" were considered interesting. This person received a total of 50 IT injections (placebo and then tofersen). An effect was not immediately detectable, but over some months, the patient noticed a stabilisation of an otherwise relatively rapidly evolving disease. The patient also reported significant improvement in using one of the hands, with the capacity to now move fingers and hold objects, which was no longer the case before. However, the patient noticed the effect to be fluctuating, as the ability of using the hand varies from one injection to the next. It was

noted by CHMP that, although helpful, the above case corresponds to a single case. Another interesting point raised was the adequacy of the dose (whether 100 mg was the highest tolerated dose or whether testing of a higher dose was limited by a volume too high for intrathecal injection).

In the view of CHMP, from a clinical point of view, prescription of tofersen should be restricted to physicians experienced in the management of ALS and administered by, or under the direction of, healthcare professionals experienced in performing lumbar punctures. Feedback from patient organisations included the question whether follow up administrations of tofersen could be performed in neurology departments, which are not specialised ALS centres in order to reduce the burden of long-distance travelling for patients if administration is restricted to specialised centres. However, as serious neuroinflammatory ADRs have been identified which partly overlap with ALS symptoms, a cautious approach is indicated.

Comprehensiveness of the data package

1. Quality of evidence (including feasibility considerations)

Despite the rarity of the disease, a randomised placebo-controlled trial was performed, which is supported. The main study was adequately designed and aimed to demonstrate superiority to placebo in the change from Study 101 Part C baseline ALSFRS-R to week 28. The duration of the study, however, was too short to confirm efficacy of tofersen treatment, also given the inherent heterogeneity in SOD1-ALS with more than 200 variants. The study failed to demonstrate a statistically significant effect in the pre-specified primary analysis such that there is no confirmatory proof of efficacy.

2. Efficacy: precision of effect size

The precision of the effect size of tofersen on the mean change from baseline in ALSFRS-R compared to placebo could not be adequately estimated. At week 28, the difference was neither statistically significant nor clinically compelling. A trend in favour of tofersen treatment was consistently shown across all endpoints. However, the size of the effect depended on what covariates were included in the analysis and what methods for missing data imputation were used. Confidence intervals were generally wide. Overall, the size of the potential treatment effect could not be precisely estimated. The effect size can also not be precisely estimated from the available long-term data due to the uncontrolled nature of these data and the increasing number of missing data over time.

3. Efficacy: clinical meaningfulness of the endpoint

Robust reductions from baseline of 60-70% in plasma and CSF NfL at week 28 though to week 104 were statistically significant. Although, neurofilament is not a validated surrogate endpoint for efficacy, it is increasingly recognised as an important biomarker in ALS and may be considered a reasonably likely surrogate endpoint in early established ALS (Benatar M et al. *Brain* 2023). Also, the majority of the SAG-N experts considered that NfL levels can be used for predicting a clinical effect in patients with ALS, at least on a population level.

ALSFR-R is an appropriate and validated scale to assess effects on functional aspects in patients with ALS. At week 28, the difference between tofersen and placebo in the change from baseline in ALSFRS-R total score was small and not statistically significant. Based on the knowledge for ALS in general, a decrease in ALSFRS-R total score is approximately 1.0 point per month and the difference observed with placebo-controlled trial was 1.2 in the prespecified mITT population and 2.1 points after post hoc analyses with baseline plasma NfL as a covariate. This small slowing of decline in clinical function is not considered clinically meaningful.

Using the data from OLE study, there were consistent trends in favour of tofersen in function, strength and QoL up to week 104.

4. Efficacy: duration of efficacy

The disease has a variable progression. It is not always a linear decline in function, but it is estimated that people living with ALS decline by an average of ~1 point per month. All participants enrolled in Study 101 Part C had a median opportunity for follow-up from baseline of 3.4 years (range: 2.2 to 3.9 years), extending beyond the 2.3-year median survival for SOD1-ALS characterized in the literature. The data suggest stabilisation or even improvement of disease in the long-term. However, the interpretation of the data beyond week 28 is challenging.

5. Safety: exposure

There was limited controlled safety exposure. The number of tofersen exposed subjects in the clinical studies (N=166) and, taking the difficulties of causality assessment in uncontrolled ALS studies into consideration, in particular the controlled exposure in the pivotal study (72 tofersen and 36 placebo patients) is rather limited.

6. Safety: length of follow-up

As of 28 February 2023, a total of 166 participants from Studies 101 and 102 were exposed to any dose of tofersen for 408.82 participant-years. The median exposure to tofersen for this group was 131.7 weeks. Participants from Parts A and B of Study 101 who received at least 1 dose of tofersen 100 mg in Study 102 have had the opportunity for a median follow-up of 4.7 years (range: 3.1 to 5.8 years) in Study 102. The length of safety follow-up is considered appropriate.

7. Target population vs study population

The study population was SOD1-ALS, which corresponds to 2% of all ALS patients. For inclusion in the study, the SOD1 mutation was confirmed, and the weakness was attributable to ALS. However, over 200 SOD1 mutations associated with ALS have been identified to date, with varying degrees of evidence about pathogenicity, disease progression rate and variable prognosis for survival. The indication is aiming for all SOD1-ALS patients, but it acknowledged that not all SOD mutations can be studied. The study population is considered sufficiently representative of the target population.

8. Pharmacological rationale

The antisense oligonucleotide, tofersen, has been developed to target the increase and accumulation of toxic SOD1 in SOD1 ALS. The hybridisation of tofersen to the cognate mRNA results in RNase-H-mediated degradation of the mRNA for SOD1, which reduces the amount of SOD1 protein synthesis and accumulation. In nonclinical studies, dose-dependent reductions in SOD1 mRNA and SOD1 protein were observed with administration of tofersen in rodents and NHPs [McCampbell 2018]. In the SOD1 G93A mouse model (i.e., with transgenic expression of G93A mutant form of human SOD1 driven by the human SOD1 promoter), tofersen drove reductions in serum pNfH, improvement in motor performance and weight loss, reversal of muscle action potential loss, and prolongation of survival.

Also, in patients with SOD1 ALS, relevant and sustained reductions in SOD1 and NfL were demonstrated. Therefore, indications of slowing of axonal injury and neurodegeneration derive from non-clinical and clinical pharmacology data.

9. Natural history/ course of the disease

Data from the literature on SOD1-ALS are limited and are continuously being generated. The applicant has provided some publications available for the natural disease course, as well as for the broader ALS condition.

In conclusion, the data are not considered comprehensive to justify a full MA.

Marketing authorisation under exceptional circumstances

As comprehensive data on the product is not available (see above), a marketing authorisation under exceptional circumstances was proposed by the CHMP during the assessment, after having consulted with the applicant.

The CHMP considers that the conditions for a marketing authorisation under exceptional circumstances are fulfilled.

Firstly, the CHMP considered that, based on the totality of the evidence, the benefit-risk balance is positive (see above).

Secondly, the CHMP considered that the applicant has sufficiently demonstrated that it is not possible to provide comprehensive data on the efficacy and safety under normal conditions of use, because the applied for indication is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence. In this respect, the CHMP is of opinion that, in order to provide comprehensive evidence on the efficacy of the medicinal product under normal conditions of use, confirmatory evidence of efficacy would be needed, generated by additional placebo-controlled study(ies) in symptomatic SOD1-ALS patients. However, it is agreed that such studies are not feasible, mainly due to the rarity of the disease (the condition to be treated, i.e. SOD1-ALS is only a small subset of ~2% of ALS and very rare) and due to the impact of tofersen's approval on patient recruitment.

Furthermore, the data that are expected to be feasible to collect (which form the basis of the imposed specific obligations), are unlikely to fully address the uncertainties above described, in particular regarding the medicine's efficacy and thus, to generate a comprehensive dataset. The main reasons are summarised below, and additional details can be found in the section "Additional data needed in the context of MA under exceptional circumstances", in section "2.6.6 Discussion on clinical efficacy" above:

- The data expected to be generated by study 233AS303 conducted with presymptomatic carriers of selected variants of SOD1 mutation is unlikely to provide confirmatory evidence of efficacy for the following reasons/limitations: (1) the sample size of 28 patients in the placebo-controlled part B is considered too small for a robust demonstration of a treatment effect; increasing the sample size is not considered feasible (please see also "Additional efficacy data needed in the context of a MA under exceptional circumstances" in section 2.6.6 of this assessment report); (2) the data collected from the remaining participants in study 303 will be uncontrolled, open-label; (3) heterogeneity in study population is still likely to be considerable although an enriched SOD1 population with the aim to increase sensitivity has been selected with a subset of 17 SOD1 variants being pre-specified for inclusion that are thought to have high or complete penetrance, informed by the scientific literature, genetic databases, and clinical experience; (4) there is uncertainty about the specific threshold of NfL for being prognostic to becoming symptomatic, with the trajectory model as the basis for the threshold being exploratory and probably lacking robustness; (5) there is also uncertainty regarding the time between meeting the threshold and developing symptoms. Hence, the design of study 303 is based on many assumptions, especially the one underlying the NfL trajectory model, and the high uncertainties regarding the validity of the assumptions mean that the study cannot be expected to be conclusive. Finally, the results of study 233AS303 in an enriched population of presymptomatic SOD1-ALS carriers cannot simply be extrapolated to the broad SOD1-ALS patient population with symptomatic disease, the target population of the indication.
- Study 233AS102 is expected to provide meaningful data in further characterising the benefits and the risks of tofersen, in particular with regard to long-term safety (primary aim) and also additional information on survival. However, due to the limitations of the design (the study is not a placebo-controlled trial; all patients will be treated (open label)), this would not generate data that would allow to provide confirmatory evidence of clinical benefits of tofersen treatment. Therefore, the results of this study are not expected to lead to a comprehensive confirmatory dataset.

- Study 233AS401 is an observational registry-based study. While the primary aim is to evaluate the long-term safety of tofersen in patients with SOD1-ALS, some efficacy data are expected to be collected as part of the secondary objective of the study (in particular disease progression measured by the ALSFRS-R, outcomes related with respiratory function and survival). However, as this is an observational, registry-based study lacking randomisation and blinding, it would not likely generate data that would allow to provide confirmatory evidence of efficacy. Therefore, CHMP concluded it is unlikely that the data generated from this study will provide a comprehensive dataset.

The CHMP acknowledged the challenges to be able to generate new data during the post-authorisation phase. It is considered that this evidence generation plan will likely provide meaningful data to further characterise the efficacy and the safety in addition to the already available information. However, because of the mentioned uncertainties and limitations of the above-described studies, the CHMP is of the opinion that the amount and the quality of the data expected to be generated is unlikely to be considered as comprehensive.

Therefore, recommending a marketing authorisation under exceptional circumstances was considered appropriate.

3.8. Conclusions

The overall benefit/risk balance of Qalsody is positive, subject to the conditions stated in section 'Recommendations'.

Divergent position(s) are appended to this report.

4. Recommendations

Outcome

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

The treatment of adults with amyotrophic lateral sclerosis (ALS), associated with a mutation in the superoxide dismutase 1 (SOD1) gene.

The CHMP therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

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

- **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.

- **Obligation to conduct post-authorisation measures**

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances.

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	**Due Date**
To further investigate the long-term efficacy and safety of tofersen in the treatment of SOD1-ALS, the MAH shall submit the final results of the long-term extension study (Study 233AS102).	by 30 September 2025
-To further investigate if initiation of tofersen in presymptomatic SOD1ALS patients can delay or even prevent emergence of clinically manifested ALS (CMALS), the MAH shall submit the final results of the phase 3 study in patients with clinically presymptomatic SOD1-ALS (Study ATLAS 233AS303).	by 31 December 2028
To further characterise variant-specific survival, the MAH will provide the final results of the descriptive integrated analyses of disease duration (survival) by SOD1 variant-type in tofersen-treated (Studies 101/102; disease registries) vs. patients untreated with tofersen (disease registries, natural history datasets/literature).	by 30 June 2027
To further evaluate the long-term safety of tofersen in patients with SOD1-ALS, the MAH shall conduct and submit the results of an observational registry-based study 233AS401 according to the agreed protocol.	Annually (with annual reassessment)
In order to ensure adequate monitoring of safety and efficacy of tofersen in the treatment of patients with SOD1-ALS, the MAH shall provide yearly updates on any new information concerning the safety and efficacy of tofersen.	Annually (with annual reassessment)

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

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that tofersen is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

Refer to Appendix on new active substance (NAS).

Divergent position(s)

Divergent position(s) to the majority recommendation are appended to this report.

DIVERGENT POSITION DATED 22 FEBRUARY 2024

The undersigned member(s) of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation under exceptional circumstances of Qalsody (tofersen) for the following indication:

Qalsody is indicated for the treatment of adults with amyotrophic lateral sclerosis (ALS), associated with a mutation in the superoxide dismutase 1 (SOD1) gene.

The reasons for the divergent opinion were the following:

The pivotal study 101 was a failed study. Efficacy results from part C (phase 3 part) of study 101 did not meet the primary study objective. While a reduction of cerebral spinal fluid SOD1 protein, implying target engagement, and a reduction in plasma neurofilament (NfL) levels, a PD marker of axonal damage, were observed, the study was not appropriately designed to demonstrate clinical efficacy (ALSFRS-R). The change in ALSFRS-R total score over 28 weeks was statistically non-significant (p=0.9689). The Company had disregarded previous EMA scientific advice that strongly recommended to extend the study duration beyond 28 weeks to allow capturing a meaningful change in clinical outcomes as measured by ALSFRS-R. NfL´s ability to predict clinical benefit still needs to be demonstrated (unvalidated surrogate endpoint biomarker, for which the link with clinical outcomes was and is not fully established). The results of the open label long term extension data from study 102, are difficult to interpret. Post hoc analyses of clinical efficacy in subgroups corrected for baseline NfL levels were not alpha corrected nor did they lead to convincing (not even nominal significant) results.

Overall, considering the lack of demonstrated clinical benefit (primary endpoint), a signal of target engagement and impact on a marker of axonal damage although its clinical relevance (i.e., surrogacy) still needs to be defined, could be grounds for granting conditional marketing authorisation (CMA) in an area of high unmet need. The CMA should allow collecting further clinical efficacy (trial or registry) data, in a way suited to definitively conclude on the positive benefit/risk balance of Qalsody.

CHMP Member(s) expressing a divergent opinion:

Peter Mol

Paolo Gasparini

DIVERGENT POSITION DATED 22 FEBRUARY 2024

The undersigned member(s) of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation of Qalsody (tofersen) for the following indication:

Qalsody is indicated for the treatment of adults with amyotrophic lateral sclerosis (ALS), associated with a mutation in the superoxide dismutase 1 (SOD1) gene.

The reasons for the divergent opinion were the following:

In the pivotal 101 trial of Qalsody, the change in ALSFRS-R total score over 28 weeks (primary endpoint) was statistically non-significant compared to placebo (p=0.9689). All further inferences of potential benefit are liable to bias and are not type 1 error controlled.

Treatment with Qalsody resulted in a reduction of CSF SOD1 protein, implying some level of target engagement. Notably, there are no external data to support what level of reduction might be required for clinical efficacy.

A treatment-dependent reduction in plasma NfL levels was demonstrated. However, the predictive value of NfL as a surrogate in ALS has not been established. The prognostic value of this biomarker may be considered limited, as instantiated by the SAG-N proceedings of Oct 30th 2023. Notably, the 101 study itself failed to corroborate the predictive value of NfL.

In conclusion, the efficacy of Qalsody in the treatment of patients with ALS associated with a mutation in the superoxide dismutase 1 (SOD1) gene has not been established.

Qalsody may be causative of serious neuroinflammatory reactions, which is not acceptable in the absence of established benefit. Moreover, treatment is burdensome for the patient, requiring repeat lumbar punctures.

The B/R balance of Qalsody is negative.

CHMP Member(s) expressing a divergent opinion:

Kristina Dunder

Maria Concepción Prieto Yerro

Outi Mäki-Ikola

Sol Ruiz