# PERIPHERAL AND CENTRAL NERVOUS SYSTEM DRUGS ADVISORY COMMITTEE

# BIOGEN BRIEFING DOCUMENT

## QALSODY® (tofersen)

## NDA# 215887

## Indication: For the Treatment of Adults With Amyotrophic Lateral Sclerosis Associated With a Mutation in The Superoxide Dismutase 1 Gene (SOD1-ALS)

## MEETING DATE: 22 MARCH 2023

## AVAILABLE FOR PUBLIC RELEASE

**Biogen MA Inc.**
225 Binney Street
Cambridge, MA 02142
United States

# TABLE OF CONTENTS

## LIST OF TABLES

# LIST OF FIGURES

# ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| ACMG | American College of Medical Genetics and Genomics |
| AE | adverse event |
| ALS | amyotrophic lateral sclerosis |
| ALSAQ-5 | Amyotrophic Lateral Sclerosis Assessment Questionnaire-5 |
| ALSFRS-R | ALS Functional Rating Scale-Revised |
| ANCOVA | analysis of covariance |
| ASO | antisense oligonucleotide |
| ATS | American Thoracic Society |
| BLQ | below limit of quantitation |
| CI | confidence interval |
| CNS | central nervous system |
| COVID-19 | coronavirus disease 2019 |
| CSF | cerebrospinal fluid |
| EAP | expanded access program |
| EMA | European Medicines Agency |
| EQ-5D | EuroQoL 5 Dimension |
| EQ-5D-VAS | EuroQoL 5 Dimension - visual analog scale |
| EQ-5D-5L | EuroQoL 5 Dimension, 5 Level Questionnaire |
| FDA | Food and Drug Administration |
| FDASIA | Food and Drug Administration Safety and Innovation Act |
| FPS | faster-progression subgroup |
| FPS (mutation/slope) | faster-progression subgroup according to SOD1 mutation and ALSFRS-R slope |
| FPS (NF-based) | faster-progression subgroup according to neurofilament levels |
| FSS | Fatigue Severity Scale |
| FVC | forced vital capacity |
| GMR | geometric mean ratio |
| GoF | gain of function |
| HHD | hand-held dynamometry |
| HR | hazard ratio |
| IND | Investigational New Drug Application |
| ISE | integrated summary of efficacy |
| ISS | integrated summary of safety |
| IT | intrathecal(ly) |
| ITT | intent-to-treat |
| JRT | joint rank test |
| LLOQ | lower limit of quantitation |
| LMN | lower motor neuron |
| LS | least square |
| MAD | multiple-ascending-dose |
| Max | maximum |
| MCID | minimally clinically important difference |
| MedDRA | Medical Dictionary for Regulatory Activities |

| | |
|---|---|
| MI | multiple imputation |
| Min | minimum |
| mITT | modified intent-to-treat |
| MRI | magnetic resonance imaging |
| mRNA | messenger ribonucleic acid |
| N/A | not applicable or not available |
| NDA | New Drug Application |
| Nf | neurofilament |
| NfL | neurofilament light chain |
| NHP | nonhuman primate |
| NMJ | neuromuscular junctions |
| OLE | open-label extension |
| PD | pharmacodynamic(s) |
| PK | pharmacokinetic(s) |
| pNfH | phosphorylated neurofilament heavy chain |
| PNS | peripheral nervous system |
| PRO-ACT | Pooled Resource Open-Access ALS Clinical Trials |
| PT | preferred term |
| PV | permanent ventilation |
| QoL | quality of life |
| SAD | single-ascending-dose |
| SAE | serious adverse event |
| SAP | statistical analysis plan |
| SD | standard deviation |
| SMA | spinal muscular atrophy |
| SOD1 | superoxide dismutase 1 |
| SOD1-ALS | amyotrophic lateral sclerosis associated with a mutation in the superoxide dismutase 1 gene |
| SPS | slower-progression subgroup |
| SPS (mutation/slope) | slower-progression subgroup according to SOD1 mutation and ALSFRS-R slope |
| SPS (NF-based) | slower-progression subgroup according to NfL levels |
| SVC | slow vital capacity |
| UK | United Kingdom |
| US | United States |
| V | version |
| WBC | white blood cell |

# 1. INTRODUCTION

This briefing document outlines the position that tofersen-driven reductions in plasma neurofilament light chain (NfL) are a surrogate endpoint for the purposes of accelerated approval of tofersen for the treatment of amyotrophic lateral sclerosis (ALS) associated with a mutation in the superoxide dismutase 1 (*SOD1*) gene (SOD1-ALS). In this regulatory context, a surrogate endpoint is a marker that is reasonably likely to predict clinical benefit but is not itself a measure of clinical benefit. Determining whether a marker is reasonably likely to predict clinical benefit is ultimately a matter of judgment based on the biological plausibility of the relationship between the disease, the endpoint, the desired effect, and the empirical evidence to support that relationship.

The support for NfL as a surrogate endpoint in SOD1-ALS stems from the following:

1. **Biological plausibility**: Neurofilaments are integral structural components of neurons and an indicator of motor neuron integrity. ALS is a disease associated with motor neuron death. Correspondingly, NfL is a strong prognostic indicator of disease progression and survival in ALS, with higher baseline NfL levels being associated with faster disease progression and shorter survival [Thompson 2022].
2. **Empirical evidence**: Tofersen-treated groups in the Phase 3 VALOR study and its open-label extension (OLE) had robust reductions in NfL that were apparent prior to the emergence of a discernable clinical benefit. Additionally, a causal inference statistical model indicates that tofersen-driven reductions in plasma NfL directly correlate with a reduction in worsening of clinical function and prolonged event-free survival/overall survival.

*SOD1-ALS*

SOD1-ALS is a serious, progressive, and uniformly fatal disease that affects approximately 330 people in the United States (US), a prevalence well below the 200,000-person statutory threshold of an orphan disease in the US [Brown 2021; Zou 2017].

In people living with SOD1-ALS, a mutation in the *SOD1* gene results in production and accumulation of a toxic form of SOD1 protein, which leads to axonal injury, neurodegeneration, and death of motor neurons, as reflected by elevated neurofilament levels [Bunton-Stasyshyn 2015]. This then leads to weakness, functional decline, and ultimately death. To date, there are no available treatments that target the underlying pathophysiology of SOD1-ALS.

Tofersen is an intrathecally (IT)-administered antisense oligonucleotide (ASO) designed to degrade SOD1 messenger ribonucleic acid (mRNA) to reduce synthesis of SOD1 protein. Reducing translation of new SOD1 protein prevents further accumulation of new toxic SOD1 and allows natural clearing mechanisms to remove existing toxic SOD1 protein. By reducing the amount of toxic SOD1 protein, tofersen preserves motor neuron integrity, as shown by reductions in neurofilament in cerebrospinal fluid (CSF) and blood. In G93A mutant mice, administration of a SOD1-lowering ASO after disease onset led to reduced levels of neurofilament and reversed loss of compound muscle action potential loss. When administered prior to disease onset, tofersen-treated mice survived longer, maintained weight longer, and achieved better motor performance than those that received control [McCampbell 2018].

*Tofersen Development Program*

The tofersen development program consists of 4 clinical studies, central to which are the Phase 3 VALOR study and its ongoing OLE. As of the 15 July 2022 data cutoff for the 120-day safety update, 147 adults with SOD1-ALS had received tofersen 100 mg for 312.56 person-years (median exposure: 2.3 years). Dose levels ranging from 10 to 100 mg were evaluated in the single- and multiple-ascending-dose study. The highest tofersen concentrations and greatest reductions in total CSF SOD1 protein, an indirect marker of target engagement, and neurofilament, a marker of axonal injury and neurodegeneration, were seen in the 100 mg group.

The 100 mg dose was moved into the Phase 3 VALOR study, a 28-week, placebo-controlled study conducted in 108 adults with SOD1-ALS. In an attempt to account for disease heterogeneity, the study was designed to enroll a broad population of adults with SOD1-ALS, while identifying a subset of individuals expected to progress quickly as the primary analysis population according to *SOD1* mutation type and prerandomization ALS Functional Rating Scale-Revised (ALSFRS-R) slope (faster-progression subgroup [FPS]; n = 60). Change in ALSFRS-R was assessed as the primary endpoint, analyzed in the FPS via the joint rank test (JRT). Changes in CSF SOD1 protein, plasma NfL, slow vital capacity (SVC), hand-held dynamometry (HHD), event-free-survival, and survival were assessed as key secondary endpoints. In retrospect, *SOD1* mutation type and prerandomization ALSFRS-R slope were poor predictors of the rate of decline of the FPS (Section 3.1.2).

The development program was also prospectively designed to combine data from VALOR and its OLE in accordance with the integrated summary of efficacy (ISE) analysis plan. This integration enabled comparison of early-start tofersen (participants who were randomized to tofersen in VALOR and continued tofersen in the OLE) versus delayed-start tofersen (participants who were randomized to placebo in VALOR and had the opportunity to initiate tofersen in the OLE approximately 6 months later). To maintain the integrity of the ongoing data collection in the OLE, individual treatment assignments from VALOR will remain blinded through completion of the study.

*Effects of Tofersen*

Administration of tofersen 100 mg leads to sustained reductions in total CSF SOD1 protein of approximately 35%. Modeling based on nonhuman primate (NHP) data and analysis of autopsy tissue from tofersen-treated participants suggest this magnitude of reduction in CSF is associated with > 90% reductions of SOD1 protein in the spinal cord (particularly relevant given that SOD1-ALS is predominantly a lower motor neuron [LMN] disease [Millecamps 2010]) and > 30% in the cortex. These reductions were apparent by approximately Week 8, consistent with the pharmacokinetics (PK) of tofersen and estimated half-life of SOD1 protein.

Robust reductions (approximately 50% to 60%) in plasma neurofilament followed, with levels reaching their new nadir by approximately Week 16. These reductions were observed across different *SOD1* mutation types, rates of disease progression, and stages of disease; consistent reductions were also seen with different matrices and analytes (CSF NfL, plasma/CSF phosphorylated neurofilament heavy chain [pNfH]).

Despite clear evidence of biological activity, a statistically significant difference was not observed on the primary analysis in VALOR at Week 28. This may have been partly related to aspects of the study design (e.g., enrichment strategy and study duration) and/or inherent

challenges in a heterogeneous disease such as SOD1-ALS as detailed in Section 2.2. However, at Week 28, consistent trends favoring tofersen were observed across measures of clinical function, respiratory function, strength, and quality of life. These effects were further supported by an apparent stabilization of weight loss in the tofersen group.

By Week 52, clear and meaningful differences favoring early-start tofersen (as compared to delayed-start tofersen) were observed across clinical outcome measures of clinical function (ALSFRS-R), respiratory strength (SVC), muscle strength (HHD), and patient-reported outcomes of disease severity and quality of life (QoL; Amyotrophic Lateral Sclerosis Assessment Questionnaire-5 [ALSAQ-5], Health Standardised Questionnaire by EuroQol Group [EuroQoL 5 Dimension, 5 Level Questionnaire [EQ-5D-5L]). These differences favoring early-start tofersen were seen regardless of which subgroup, statistical methodology, and/or covariates were incorporated. As of the 16 January 2022 data cutoff, all participants enrolled in VALOR had the opportunity for at least 1 year of follow-up (median: 2.3 years; range: 1 to 2.8 years). Despite this duration of follow-up, there were a limited number of death-equivalent events, thus precluding estimation of the median time to event in both treatment groups. The risk of death or permanent ventilation (PV) was reduced by 64%, and the risk of death was reduced by 73% in the early-start group compared to the delayed-start group.

While these analyses are considered largely exploratory, the effects seen were consistent across measures and followed a temporal relationship with strong biological plausibility (e.g., CSF SOD1 lowering followed by neurofilament reductions preceding discernable clinical benefit). In a disease associated with progressive decline, evidence of improvement in strength and function seen in some tofersen-treated participants is completely inconsistent with the natural history. Taken together, the probability of these observations being due to chance is very low. These data provide important clinical context regarding the relationship between reductions in neurofilament and clinical benefit over time.

This relationship is further supported by a statistical model built upon data from VALOR and its OLE, which illustrates that early tofersen-driven reductions in plasma NfL are directly associated with a slowing in decline in clinical function, strength, and quality of life and a reduced risk of death-equivalent events over time.

*Tofersen-Driven Neurofilament Reductions as a Surrogate Biomarker That is Reasonably Likely to Predict Clinical Benefit in SOD1-ALS*

Neurofilaments are intermediate filaments uniquely expressed in neurons. When axons are injured or degenerating, neurofilament leaks into the interstitial fluid before passing into the CSF and blood where levels can be quantified [Gaetani 2019; Khalil 2018].

The ALS literature consistently demonstrates that neurofilament levels are prognostic for disease progression and survival, with higher levels associated with faster progression and shorter survival [Abu-Rumeileh 2020; Brettschneider 2006; De Schaepdryver 2020; Falzone 2022; Lu 2015; Rossi 2018; Thompson 2022; Thouvenot 2020; Vacchiano 2021; Zetterberg 2007].

Given that SOD1-ALS is driven by motor neuron loss, with faster-progressing disease associated with higher levels of neurofilament, lowering of neurofilament is thought to represent a slowing of axonal injury and neurodegeneration, thus providing objective evidence of treatment effect. That said, neurofilament reductions may not be observed with all effective ALS therapies. For example, a therapy targeting something other than neurodegeneration (e.g., a therapy targeting

muscle contraction or neuromuscular transmission) would not be expected to lower neurofilament.

Consistent with its mechanism, which targets the underlying upstream cause of SOD1-ALS, tofersen administration led to reductions in neurofilament that were sustained over time. These reductions, which appeared to be maximized by approximately 16 weeks, preceded evidence of effects on clinical function, respiratory function, strength, quality of life, and survival, which were not clearly discernable until Week 52 and beyond.

Furthermore, the statistical model built using data from VALOR and its OLE demonstrates that early (Week 16) tofersen-driven reductions in plasma NfL are directly associated with a reduction in worsening of clinical function (at Week 28) and prolonged event-free survival/overall survival over time.

Taken together, data from the literature and the tofersen program support that treatment-driven reductions in neurofilament are reasonably likely to predict clinical benefit in SOD1-ALS in the context of an accelerated approval.

*Confirmatory Evidence Generation Plan*

If approved through the accelerated approval pathway, Biogen will provide confirmatory data to inform on the clinical benefit of tofersen, as expeditiously as possible. Confirmation of clinical benefit could come from the currently enrolling ATLAS study (Study 233AS303; NCT04856982). ATLAS is an ongoing, adequate, and well-controlled trial that is designed to evaluate the effects of tofersen when initiated in *SOD1* mutation carriers with biomarker evidence of disease activity (elevated plasma NfL) but without clinically manifest disease [Benatar 2022]. Initiated in mid-2021, over 50% of the target population (n = 150) has been enrolled at sites across 14 countries to date. Based on the current study design and enrollment rate, data from this study are expected in 2027. Additional long-term data generation plans to supplement the ATLAS study include the combined analysis of final data from VALOR and its OLE to evaluate the effects of early-start versus delayed-start tofersen on survival and function and descriptive analyses of disease duration by *SOD1* variant type in tofersen-treated versus untreated individuals based on real-world evidence.

## 1.1. Proposed Indication

The indication proposed by Biogen is as follows: *tofersen is indicated for the treatment of adults with amyotrophic lateral sclerosis associated with a mutation in the superoxide dismutase 1 (SOD1) gene (SOD1-ALS).*

## 1.2. Product Description

Tofersen is a 20-base residue (20-mer) 5-10-5 2′-methoxyethyl gapmer mixed backbone oligonucleotide. Tofersen is administered IT via lumbar puncture at a dose of 100 mg/15 mL. Treatment with tofersen is initiated with 3 loading doses of 100 mg administered once every 2 weeks, followed by a maintenance dose of 100 mg once every 4 weeks.

## 1.3. Regulatory History

The Investigational New Drug Application (IND) was cleared to proceed in November 2015, granted fast track designation in December 2015, and orphan drug designation in September 2016.

The tofersen clinical development program was designed with input from the Food and Drug Administration (FDA) through a series of formal meetings and in line with the FDA's ALS guidance document [FDA 2019]:

- **January 2015**: Biogen sought pre-IND advice from the FDA on the design of nonclinical toxicology studies to support conditions of safe use in clinical trials.
- **June 2017**: Biogen met with the FDA in a Type C meeting to obtain feedback on the clinical development strategy for tofersen in SOD1-ALS.
- **May 2019**: Biogen met with the FDA in a Type C meeting and discussed the proposed approach for enriching the primary analysis population for the planned Study 101 Part C (VALOR) based on *SOD1* mutation type and prerandomization ALSFRS-R slope.
- **June 2020**: the FDA denied Biogen's request for Breakthrough Therapy designation.
- **January 2021**: Biogen received preliminary feedback from the FDA after a Type C meeting request to discuss Biogen's statistical analysis strategy for VALOR.
- **August 2021:** Biogen submitted the final statistical analysis plan (SAP) to the IND ahead of final database lock for VALOR.
- **September 2021:** Biogen met with the FDA in a Type C meeting to discuss the top line data of the VALOR final analysis.
- **December 2021:** Biogen met with the FDA in a Type C meeting to discuss additional data available post-top line data read out and the potential viability of a new drug application under the traditional approval pathway.
- **April 2022**: Biogen met with the FDA in a Type B pre-New Drug Application (NDA) meeting to discuss the format and contents of the planned NDA and the utility of NfL as a surrogate endpoint that is reasonably likely to predict clinical benefit in the context of an NDA seeking approval under the accelerated approval pathway.
- **May 2022**: the NDA seeking approval for tofersen under the accelerated approval pathway was submitted to the FDA and subsequently filed by the FDA in July 2022 as a Priority Review.

## 1.4. Accelerated Approval

As referenced in the FDA guidance [FDA 2014] and associated regulations, the accelerated approval provisions of the Food and Drug Administration Safety and Innovation Art (FDASIA) in section 506I of the Federal Food, Drug, and Cosmetic Act provide that the FDA may grant accelerated approval to the following:

*. . . a product for a serious or life-threatening disease or condition . . . upon a determination that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical*

*benefit, or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality, that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments.*

The FDASIA provides that FDA has the authority to consider pharmacologic or other evidence developed using biomarkers or other scientific methods or tools, in conjunction with other data, in determining whether an endpoint is reasonably likely to predict clinical benefit. By indicating that the FDA should take into account, ". . . the severity, rarity, or prevalence of the condition . . ." in considering whether to grant an accelerated approval, the FDASIA reinforces the FDA's longstanding commitment to regulatory flexibility regarding the evidence required to support product approval for the treatment of serious or life-threatening diseases with limited therapeutic options.

Of note, the FDA's ALS guidance document [FDA 2019] encourages sponsors to incorporate exploratory biomarkers in all phases of development of ALS drugs and states that, in the future, greater scientific understanding of ALS may provide opportunities for discussion of surrogate endpoints that are reasonably likely to predict clinical benefit and that might serve as a basis for accelerated approval.

For purposes of accelerated approval, a surrogate endpoint is a marker, such as a laboratory measurement, radiographic image, physical sign, or other measure, that is thought to predict clinical benefit but is not itself a measure of clinical benefit. A "reasonably likely" surrogate endpoint for support of an accelerated approval does not yet have sufficient evidence to be a validated surrogate endpoint (which may support full, or traditional, approval). Determining whether an endpoint is reasonably likely to predict clinical benefit is a matter of judgment that will depend on the biological plausibility of the relationship between the disease, the endpoint, and the desired effect, and the empirical evidence to support that relationship.

To support an accelerated approval, Biogen would be required to conduct a study to confirm clinical benefit. If the confirmatory trial shows that the drug provides a clinical benefit, the FDA may grant a traditional approval for the drug. If the confirmatory trial does not show that the drug provides clinical benefit, the FDA has regulatory procedures in place that could lead to removing the drug from the market.

# 2. BACKGROUND

## 2.1. Overview of Amyotrophic Lateral Sclerosis

ALS is a rare, progressive, and ultimately fatal neurodegenerative disease that causes loss of upper motor neurons and LMNs within the cortex, brainstem, spinal cord, and peripheral nervous system (PNS) [Bunton-Stasyshyn 2015; Wijesekera and Leigh 2009]. 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) [Brown and Al-Chalabi 2017; Bunton-Stasyshyn 2015; Lechtzin 2018]. ALS may present in any anatomical region and spread throughout the body with variable speeds and patterns in different people living with the disease.

A recent systematic review and meta-analysis study reports pooled prevalence rates (per 100,000 persons) and incidence rates (per 100,000 person-years) as 6.22 and 2.31 for Europe, 5.20 and 2.35 for North America, 3.41 and 1.25 for Latin America, 3.01 and 0.93 for Asian countries excluding Japan, and 7.96 and 1.76 for Japan, respectively [Brown 2021]. The global prevalence of ALS is estimated to be approximately 4.42 per 100,000 persons [Xu 2020].

## 2.2. Overview of SOD1-ALS

In a subset of people living with ALS, the disease is caused by mutations in the gene encoding the enzyme SOD1 (SOD1-ALS). The *SOD1* gene encodes an abundant dimeric enzyme, copper/zinc superoxide dismutase (Cu/Zn SOD or SOD1), which catalyzes the transmutation of superoxide ($O_2^-$) into oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$) [McCord and Fridovich 1969].

In people living with SOD1-ALS, mutations in the *SOD1* gene lead to production and accumulation of a toxic form of SOD1 protein via a gain of function (GoF) mechanism [Bunton-Stasyshyn 2015]. The presence of this toxic protein is thought to drive axonal injury and neuronal degeneration with subsequent leakage of neurofilament into the CSF and blood.

SOD1-ALS affects approximately 2% of the ALS population, with an estimated prevalence in the US of approximately 330 people [Brown 2021; Zou 2017]. SOD1-ALS is a predominantly LMN disease [Millecamps 2010], and as with all cases of ALS, it is associated with progressive decline in strength and function.

Over 200 causative *SOD1* mutations associated with ALS have been identified to date [ALSoD 2023]. 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 [Robberecht and Philips 2013; Rosen 1993].

The natural history of SOD1-ALS is highly variable and generally poorly characterized due to the rarity of the disease and limited data availability. A retrospective cohort study that reviewed records from 175 people living with SOD1-ALS across 15 institutions in North America [Bali 2017; Coppedè 2018] described a mean (± standard deviation [SD]) age of onset (49.7 ± 12.3 years) generally similar to that in the broad ALS population, though this can vary meaningfully across individual *SOD1* gene mutation types [Bali 2017]. The rapidity of disease progression also varies substantially across *SOD1* mutation types, with disease durations ranging from less than a year to more than 20 years [Bali 2017; Cudkowicz 1997]. For example, the p.Ala5Val

(A5V; A4V) mutation, the most prevalent variant in North America, was associated with a median survival at or below 1.2 years (based on KM estimates) and mean disease duration of 1.4 ± 0.7 years. In contrast, the p.Gly42Asp (G42D; G41D) mutation has a mean disease duration of 23.5 ± 14.0 years [Bali 2017].

Despite the heterogeneity in disease progression, the underlying pathophysiology of the disease, attributable to production of toxic SOD1 protein, is thought to be consistent across GoF *SOD1* mutation types.

## 2.3. Rationale for Development of Tofersen for SOD1-ALS

### 2.3.1. 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 (hybridization). This hybridization of tofersen to the cognate mRNA results in ribonuclease-H-mediated degradation of the mRNA for SOD1, which reduces the synthesis and accumulation of SOD1 protein (both toxic and wild-type).

SOD1-ALS occurs because of the accumulation of toxic SOD1 protein in motor neurons, resulting in motor neuron death and degeneration. Tofersen binds to and degrades SOD1 mRNA, reducing the production of new SOD1 protein and preventing the accumulation of additional toxic SOD1 in motor neurons. Preventing the formation of new toxic SOD1, combined with the natural clearing mechanism to remove existing toxic SOD1, is expected to preserve motor neuron integrity (or prevent motor neuron degeneration), ultimately reducing the levels of neurofilament released into CSF and blood.

As tofersen reduces levels of the substrate for protein translation, an event that is upstream of toxic mechanisms implicated in SOD1-ALS (all of which occur at the post-translation stage), it is anticipated to provide a therapeutic benefit for all people living with SOD1-ALS, regardless of mutation type.

### 2.3.2. Evidence of Target Engagement

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 nonclinical and clinical studies. Notably, available assays including the immunoassay utilized in the tofersen development program (Covance Laboratories Inc., Limited Liability Company) are not specific to toxic SOD1 protein and likely reflect a measure of primarily native SOD1 protein. Given this, levels of total CSF SOD1 are not differentiated between people living with SOD1-ALS and healthy controls and do not correlate with disease progression over time [Winer 2013]. Therefore, a reduction in total CSF SOD1 is suitable as an indirect measure of target engagement but is not expected to predict clinical effect of tofersen.

In G93A mutant mice, administration of a SOD1-lowering-ASO after disease onset lowered serum pNfH and reversed of compound muscle action potential loss [McCampbell 2018]. When administered prior to disease onset, G93A mutant mice that received tofersen maintained weight and motor performance and survived longer [McCampbell 2018].

In clinical studies, tofersen administration led to clear and sustained reductions in SOD1 protein. In VALOR, reductions in total CSF SOD1 became apparent by approximately Week 8,

consistent with the PK of tofersen and the estimated half-life of SOD1 protein (each approximately 1 month). At Week 28 in the intent-to-treat (ITT) population, reductions in total CSF SOD1 protein of approximately 35% (geometric mean ratio [GMR] to baseline) in the tofersen group and a decrease of approximately 2% in the placebo group were observed (difference in GMRs for tofersen to placebo: approximately 34%; nominal $p < 0.0001$).

The relationship between tofersen exposure and SOD1 response was detected and characterized quantitatively by a population PK/pharmacodynamics (PD) model based on clinical data. Modeling based on NHP data suggests that this magnitude of reduction in CSF reflects a reduction in total SOD1 protein of > 90% in the spinal cord (the most relevant tissue, since SOD1-ALS is predominantly an LMN disease [Millecamps 2010]) and > 30% in the cortex.

## 2.4. Unmet Medical Need

Despite the availability of 3 approved therapies in the US (riluzole, edaravone, and sodium phenylbutyrate/taurursodiol), there remains a major unmet medical need for effective therapies that preserve function and/or prolong life for people living with ALS.

Riluzole, the most widely approved ALS treatment, received its first global approval for treatment of ALS in 1995 (US) based on 2 studies demonstrating that the time to tracheostomy or death was approximately 3 months longer in participants receiving riluzole than in those receiving placebo. Although riluzole improved survival in both studies, no effect on motor function, lung function, or strength was observed [Miller 2002]. The mode of action of riluzole is unknown, but its pharmacological activities include inhibition of glutamate release, inactivation of voltage-dependent sodium channels, and interference with intracellular events that follow neurotransmitter binding at excitatory amino acid receptors.

Edaravone received its first global approval for the treatment of ALS in 2015 (Japan) based on a single study in Japanese participants with ALS of Grade 1 or 2 in the Japan ALS Severity Classification. After 24 weeks of treatment, the average decline in ALSFRS-R scores was 2.49 points less in edaravone-treated participants than in those treated with placebo. No effect was observed on survival. A study conducted in a more progressed subset of the population did not reproduce a statistically significant effect with administration of edaravone [Writing Group and Edaravone (MCI-186) ALS 19 Study Group 2017]. The mechanism of action of edaravone in ALS is unknown.

Sodium phenylbutyrate/taurursodiol (AMX0035; tradename Relyvrio) was recently approved in Canada (known as sodium phenylbutyrate/ursodoxicoltaurine; Albrioza) and the US for the treatment of ALS. Sodium phenylbutyrate/taurursodiol is under review in Europe. In a 6-month randomized Phase 2 study conducted in the US, participants who received a combination of sodium phenylbutyrate and taurursodiol lost 2.32 fewer points on ALSFRS-R over 24 weeks than those who received placebo [Paganoni 2020]. The mechanism of action of sodium phenylbutyrate in ALS is unknown, but its pharmacological activity includes histone deacetylase inhibition and upregulation of heat shock proteins. The mechanism of action of taurursodiol in ALS is also unknown, but its pharmacological activity includes reduction of mitochondrial permeability. The ongoing, Phase 3 PHOENIX study is designed to evaluate the benefit/risk of sodium phenylbutyrate/taurursodiol in a larger and longer (48 weeks) trial (EudraCT: 2021-000250-26).

To date, no treatments designed to target the underlying pathophysiology of SOD1-ALS are available.

## 2.5. Overview of the Clinical Development Plan for Tofersen in SOD1-ALS

*Throughout this document, Study 233AS101 will be referred to as Study 101, with Study 101 Part C being referred to as VALOR. Study 233AS102 will be referred to as the OLE.*

The tofersen clinical development program comprises 2 completed studies (Study 101 Parts A, B, and C [VALOR] and Study 233HV101) and 2 ongoing studies (Study 102 [OLE] and Study 233AS303 [ATLAS]), as summarized in Table 1. As of the 16 January 2022 data cutoff for the NDA submission, 175 individuals with ALS (166 with ALS, of which 162 have SOD1-ALS) had received at least 1 dose of tofersen. These participants ranged in age from 21 to 78 years at tofersen initiation.

The development program was prospectively designed to evaluate crossover from Study 101 to the OLE in accordance with the ISE analysis plan. For VALOR participants, this integration enabled comparison of early-start tofersen (participants who were randomized to tofersen in VALOR and continued tofersen in the OLE) versus delayed-start tofersen (participants who were randomized to placebo in VALOR and had the opportunity to initiate tofersen in the OLE approximately 6 months later).

Data from VALOR and integrated analyses of data from VALOR and the OLE serve as the primary basis for evaluation of the benefit/risk of tofersen. The VALOR study was completed on 15 July 2021.

The primary data cutoff of the OLE for the integrated analyses supporting the NDA submission was 16 January 2022, coinciding with the latest interim efficacy analysis of the OLE. PK, PD, and efficacy data available as of 16 January 2022 are described herein. A later data cutoff for safety and immunogenicity data was performed on 15 July 2022, to support the 120-day safety update.

An overview of the individual studies is given in the following subsections.

## Table 1: Tofersen Clinical Development Program

| Study Identification<br>Phase<br>Status | Study Design | Primary Objective | Number of Participants Enrolled |
|---|---|---|---|
| **Studies in participants with SOD1-ALS (weakness attributable to ALS and a confirmed *SOD1* mutation)** | | | |
| Study 101 Part A<br>Phase 1/2<br>Complete | Randomized, double-blind, placebo-controlled, single-ascending-dose (SAD) study in participants with ALS[a]. | Safety, tolerability, and PK | Tofersen: 15<br>Placebo: 5 |
| Study 101 Part B<br>Phase 1/2<br>Complete | Randomized, double-blind, placebo-controlled, multiple-ascending-dose (MAD) study in participants with SOD1-ALS. | Safety, tolerability, and PK | Tofersen: 38<br>Placebo: 12 |
| Study 101 Part C (VALOR)<br>Phase 3 (pivotal)<br>Complete | Randomized, double-blind, placebo-controlled study in participants with SOD1-ALS. | Efficacy | Tofersen: 72<br>Placebo: 36 |
| Study 102 (OLE)<br>Phase 3<br>Ongoing | OLE [b] for participants who completed Study 101. | Long-term safety and tolerability | Tofersen: 139 |
| **Study in healthy volunteers** | | | |
| Study 233HV101<br>Phase 1 (radiolabeled tofersen)<br>Complete | Open-label, 4-cohort, single-dose radiolabeled tofersen ($^{99m}$Tc-MAG3-BIIB067) co-administered with unlabelled tofersen in healthy participants. | ASO distribution in the central nervous system | Tofersen: 8[c] |
| **Study in Presymptomatic *SOD1* Mutation Carriers** | | | |
| Study 233AS303 (ATLAS)<br>Phase 3<br>Ongoing | Randomized, placebo-controlled study with a longitudinal natural history run-in and open-label extension, for clinically presymptomatic adults with a confirmed *SOD1* mutation. | Efficacy | Part A: 150 (planned)[d]<br><br>Part B: 28 (planned)[e] |

[a] A total of 6 participants in Study 101 Part A did not have an *SOD1* mutation, since this was not required prior to Protocol Version 2.0. None of these 6 participants enrolled in the OLE; 2 participants received only placebo, while 4 participants received a single dose of tofersen.

[b] A blinded loading dose period was incorporated in the OLE for participants who enrolled after completing VALOR; blinded placebo or tofersen was administered at Day 15 for those who received tofersen or placebo in VALOR, respectively.

[c] Five 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 analyzed.

[d] Study 233AS303 will enroll n = 150 participants in the observational run-in period (Part A) of the study, of which approximately 34 participants (approximately 28 in Part B/C and approximately 6 in Part D) will enter the interventional study period.

### 2.5.1. Study 101

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, the protocol was amended after review of Phase 1 data from Part B of Study 101 to include a Phase 3 component (Part C; VALOR). VALOR was designed to establish the benefit/risk profile of tofersen 100 mg administered to ALS participants with a confirmed *SOD1* mutation. After completion of Study 101 (SAD, MAD, or VALOR), participants had the opportunity to move into the OLE study described below.

#### 2.5.1.1. Part A and B

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 on study and were then eligible to be screened for Part B (MAD). Participants who did not meet eligibility criteria for Part B were offered the opportunity to enroll in an ongoing OLE study.

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 enroll in the OLE.

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. It was predicted that 100 mg tofersen would achieve steady-state exposures in the cortex at a level that would be sufficient for a meaningful CSF SOD1 reduction of 25% to 30%.

#### 2.5.1.2. Part C (VALOR)

VALOR was a Phase 3, randomized, double-blind, and placebo-controlled study designed to assess the efficacy and safety of tofersen 100 mg versus placebo over 6 months. Although this study was initially designed to enroll 60 participants, and subsequently amended to enroll 99 participants after receipt of 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. Specifically, IT bolus injections of tofersen 100 mg or placebo were administered as 3 loading doses 2 weeks apart followed by maintenance doses every 4 weeks thereafter.

The prespecified primary analysis population, or protocol-defined faster-progression subgroup, also referred to as the modified intent-to-treat (mITT) population, was composed of the subset (n = 60) of participants who met the prognostic enrichment criteria for rapid disease progression based on their *SOD1* mutation type and prerandomization ALSFRS-R slope. Throughout this document, FPS (mutation/slope), where FPS stands for faster-progression subgroup, is used to

refer to the primary analysis population. To understand the effects of tofersen across the SOD1-ALS population, individuals expected to progress more slowly were also enrolled and made up the slower-progression subgroup (mutation/slope) [n = 48], also referred to as the nonmITT population. The protocol-defined slower-progression subgroup (SPS) will herein be referred to as SPS (mutation/slope).

The primary endpoint was change from baseline to Week 28 in ALSFRS-R total score. Key secondary endpoints in order of hierarchical testing included total CSF SOD1 protein, plasma NfL, percent-predicted SVC, HHD megascore, time to death or PV, and time to death.

### 2.5.2. Study 102 (OLE)

Study 102, or the OLE, is an ongoing study to assess the long-term effects of tofersen in participants who previously completed Study 101 (SAD, MAD, or VALOR). Participants will have the opportunity to be followed in the OLE for approximately 3 to 7 years, depending on timing of enrollment. While participants from Parts A and B of Study 101 had washout periods between studies and received varying dose levels depending on when they rolled into the OLE, dosing was continuous between studies for VALOR participants. The Week 28 visit in VALOR was the Day 1 visit in the OLE. To maintain the blind of Study 101, a blinded loading dose period was incorporated in the OLE, followed by open-label treatment with tofersen.

Interim data cutoffs of the OLE were performed on 16 July 2021, at the time that VALOR completed, and 16 January 2022, when all participants from VALOR had the opportunity for at least 1 year of follow-up. A further data cutoff of safety data for the OLE was taken on 15 July 2022 for the purpose of reporting the 120-day safety update to the FDA.

To protect the integrity of ongoing data collection in the OLE, study participants, site staff, and the firewalled study team remain blinded to individual treatment assignments from Study 101 through completion of the OLE, expected in mid-2024.

### 2.5.3. Study 233HV101

Study 233HV101 was a Phase 1 study in healthy participants to assess the safety, tolerability, and distribution of trace amounts of radiolabeled tofersen ($^{99m}$Tc-MAG3-BIIB067) co-administered with a single dose of unlabelled tofersen to inform the distribution of tofersen in the central nervous system (CNS).

### 2.5.4. Study 233AS303 (ATLAS)

Study 233AS303, or ATLAS, is an ongoing, Phase 3, randomized, placebo-controlled, 4-part study designed to evaluate whether tofersen, when initiated in presymptomatic carriers of an *SOD1* mutation with biomarker evidence (plasma NfL) of disease activity, can halt or delay emergence of clinically manifest ALS. Approximately 150 presymptomatic at-risk carriers of 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 surpasses the protocol-defined threshold (44 pg/mL and at least a 10 pg/mL increase from baseline) and remain clinically presymptomatic will have the opportunity to enroll in the interventional, placebo-controlled study period (Part B; n = 28). Participants in Part B who experience 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 emergence

of clinically manifest ALS prior to randomization in Part B will be eligible to screen for the open-label interventional period (Part D) for up to 2 years.

Based on the current study design and enrollment rate, data from this study are expected in 2027.

### 2.5.5. Expanded Access Program

An expanded access program (EAP) was implemented for rapidly progressing individuals with SOD1-ALS in July 2021. Biogen expanded eligibility to include all individuals with SOD1-ALS as of October 2021.

## 2.6. Discussion of Key Study Design Elements and Statistical Analysis of VALOR and OLE

### 2.6.1. Key Study Design Elements

#### 2.6.1.1. Study Population

Weakness attributable to ALS, as assessed per the discretion of the Investigator, was a key eligibility criterion for enrollment in Study 101.

For VALOR, all participants underwent centralized genetic testing and were required to have an *SOD1* mutation independently classified by the central laboratory (Prevention Genetics) as "Pathogenic" or "Likely Pathogenic," in accordance with American College of Medical Genetics and Genomics (ACMG) guidelines [Richards 2015]. Independent of Biogen, variants (e.g., those classified as being of "Uncertain Significance") could be reclassified by Prevention Genetics in accordance with ACMG variant classification guidelines (e.g., based on additional literature or evidence of segregation within the family).

In VALOR, concomitant use of riluzole and/or edaravone was allowed assuming the participant was on a stable dose for at least 30 or 60 days prior to Day 1, respectively, and was expected to remain on that dose through end of study. Randomization was stratified within each of the subgroups for disease progression based on the use of edaravone and the use of riluzole at Baseline.

An SVC criterion was also included in the eligibility criteria of VALOR to minimize the impact of potential complications of later-stage disease on interpretation of results). Specifically, participants were required to have a sitting SVC of at least 50% [≥ 65% in the FPS (mutation/slope)] the predicted value as adjusted for sex, age, and height at Screening.

#### 2.6.1.2. Mechanisms to Control for Disease Heterogeneity

To account for the significant disease heterogeneity in SOD1-ALS and the relatively short study duration of VALOR, the protocol included a primary analysis population (FPS [mutation/slope]) expected to progress quickly in the absence of an effective therapy. This population was defined according to *SOD1* mutation type and prerandomization ALSFRS-R slope (Table 2). This FPS (mutation/slope) informed the sample size calculation and served as the primary analysis population for formal testing of the primary and key secondary endpoints for VALOR.

All other participants were classified as the SPS (mutation/slope), which was expected to decline more slowly over the study period, thus offering less opportunity to demonstrate a difference

between groups. Therefore, total CSF SOD1 protein was formally tested as the primary endpoint in the SPS (mutation/slope), with only descriptive analyses performed for other endpoints.

Descriptive analyses were also performed in the ITT population, defined as all participants randomized and dosed.

**Table 2: Disease Progression Subgroups Defined According to Mutation and ALSFRS-R Slope for the Primary and Secondary Analyses of VALOR**

| | Faster-Progression Subgroup (FPS; mutation/slope) | Slower-Progression Subgroup (SPS; mutation/slope) |
|---|---|---|
| **Mutation Type And Prerandomization ALSFRS-R Slope** | Protocol-defined *SOD1* mutation historically associated with shorter survival[a] and ≥ 0.2 points/month prerandomization slope<br><br>OR<br><br>Another *SOD1* mutation and ≥ 0.9 points/month prerandomization slope | Another *SOD1* mutation and < 0.9 points/month prerandomization slope |
| **SVC Cutoff** | ≥ 65% predicted | ≥ 50% predicted |

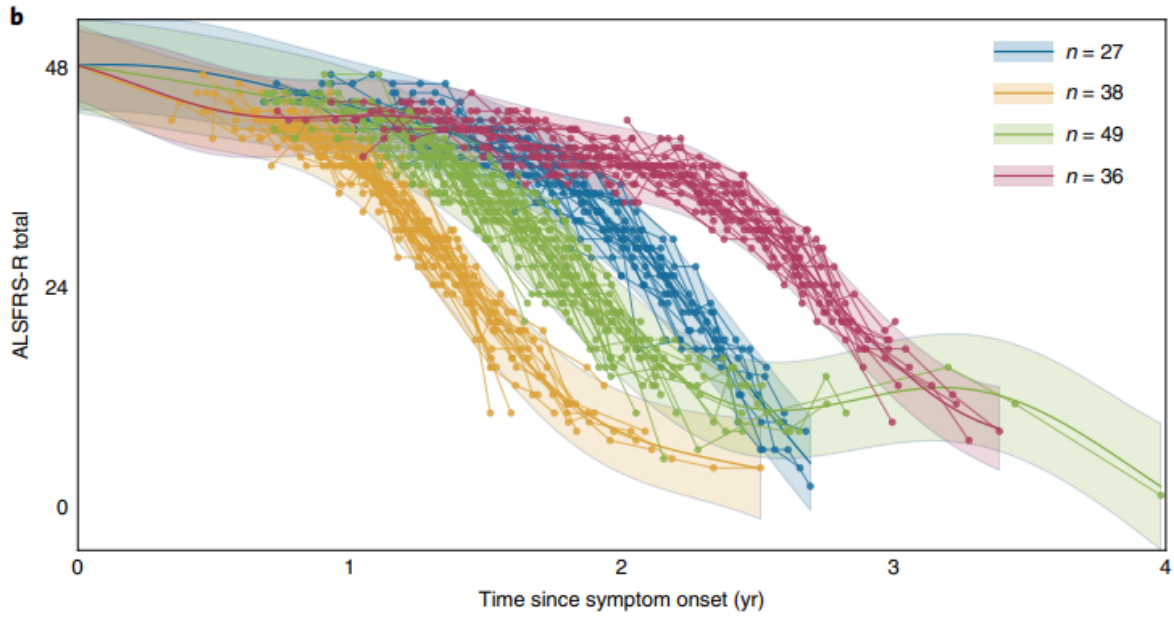
[a] p.Ala5Val, p.Ala5Thr, p.Leu39Val, p.Gly42Ser, p.His44Arg, p.Leu85Val, p.Gly94Ala, p.Leu107Val, and p.Val149Gly.

At the time of study design, *SOD1* mutation type and prerandomization ALSFRS-R slope were thought to be appropriate tools to control for disease heterogeneity at Baseline. Since then, it has become better recognized that nonlinear progression on ALSFRS-R and intra-mutation variability confound the predictive value of these measures as follows:

***SOD1* mutation type** was incorporated into the enrichment criteria as it can, in some cases, differentiate between very quickly progressing and slowly progressing phenotypes (e.g., A5V vs. G42D) over time. However, within a single mutation type, there remains significant variability in disease progression among individual carriers of the mutation. Furthermore, individuals may experience variability in the rate of progression over their disease course. These additional sources of variability limit the prognostic utility of *SOD1* mutation type over a short study period. For example, A5V mutation carriers uniformly experience rapid disease progression at some stage during their approximately 0.1- to 4-year disease course, but the timepoints during which the rapid progression occurs vary from individual to individual.

**Prerandomization ALSFRS-R slope** was incorporated into the enrichment criteria based on experience from the dexpramipexole EMPOWER study (NCT01281189, N = 943, broad ALS population), which demonstrated that participants with a prerandomization ALSFRS-R slope decline of at least 0.9 points/month maintained a mean postrandomization slope of at least 0.9 points decline per month at 6, 9, and 12 months [Cudkowicz 2013]. However, nonlinear progression on ALSFRS-R with periods of stable disease preceded or followed by periods of rapid decline [Proudfoot 2016; Ramamoorthy 2022] can limit the utility of prerandomization ALSFRS-R slope as a prognostic marker of disease progression (Figure 1) [Thompson 2022].

**Figure 1: Nonlinear Clusters From PRO-ACT Database (Repository of Merged ALS Clinical Trials Data)**




A subset of nonlinear clusters from Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) visualized; n indicates the number of individuals with ALS per cluster. The clusters were identified based on a mixture of Gaussian processes to identify individuals sharing similar disease progression patterns. The shaded area indicates the 0.95 CI. yr: year.
Source: from [Ramamoorthy 2022].

Prior to completion of VALOR and analysis of the data, it was appreciated that intra-mutation variability and nonlinear decline on ALSFRS-R could confound the prognostic value of these measures, particularly over a short study period.

Given the extensive literature supporting neurofilament as a prognostic biomarker of disease progression [Thompson 2022], it was also considered that incorporation of baseline neurofilament into the planned analysis may more effectively control for heterogeneity of disease progression.

Therefore, in addition to the planned primary analysis in subgroups based on mutation type and slope, we also prespecified analyses in alternative disease progression subgroups defined according to baseline plasma NfL levels whereby those with a baseline plasma NfL level greater than the median comprised the faster-progression subgroup (NF-based), also referred to as FPS (NF-based), and those with a baseline plasma NfL level less than the median, the slower-progression subgroup (NF-based), also referred to as SPS (NF-based).

Recognizing that adjustment for baseline NfL as a continuous covariate provides greater precision to the estimated treatment difference than categorical subgrouping based on the median, sensitivity analyses incorporating baseline plasma NfL as a covariate in the analysis of covariance (ANCOVA) model were also prespecified for the FPS (nutation/slope) and SPS (mutation/slope) subgroups. These neurofilament-based analyses were planned and prespecified in the statistical analysis plan (VALOR SAP Version [V] 2.0) prior to analysis of data from VALOR.

Applying learnings from these original analyses of the VALOR dataset, the integrated efficacy analysis plan for VALOR and its OLE was amended prior to analysis of the 16 January 2022 data cutoff to include covariate adjustment for baseline levels of NfL in analyses of the full ITT population (ISE SAP V 3.0, 02 February 2022).

#### 2.6.1.3. Study Duration

Given the urgent unmet medical need, the tofersen development program aimed to identify the earliest opportunity to detect a clinically meaningful benefit.

VALOR was designed under the assumption that a rapidly progressing subgroup of participants could be reliably identified, in which differentiation from placebo could be observed over a relatively short study period. The sample size for the VALOR primary analysis population (i.e., FPS [mutation/slope]) was determined based on available natural history data, including data from placebo-treated participants in the tofersen Phase 1 multiple-ascending-dose study (Part B; [Miller 2020]) and a randomized, placebo-controlled clinical trial of arimoclomol [Benatar 2018a]. Specifically, data from 12 participants across these datasets who matched the FPS (mutation/slope) criteria (according to *SOD1* mutation type and prerandomization ALSFRS-R slope) informed the assumption for decline on ALSFRS-R over 6 months in the placebo group (24.7-point decline in the placebo group, i.e., 3.83 points/month decline [SD of 20.39, i.e., 3.166 points/month]) that was used to calculate the sample size. Assumptions for overall survival incorporated in the sample size estimate (82% in the placebo group and 90% in the tofersen group) were based on survival estimates in the literature for A5V mutation carriers, the most prevalent fast progressing mutation type that was consistently associated with a median disease duration at or less than 1.2 years [Bali 2017]. With the above assumptions, the study was powered at 84% with 2-sided alpha of 0.05.

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

Furthermore, a study that is too short may be susceptible to an imbalance in deaths due to chance, unrelated to the disease or therapy. This is particularly impactful when analyzing the ALSFRS-R total score via the JRT. Only 1 death was observed in VALOR, which occurred in the tofersen arm and was considered unrelated to ALS disease progression and study drug.

The 6-month study duration also underestimated the time needed to maximize the biological activity (approximately 8 weeks to discernible CSF SOD1 protein reductions and approximately 16 weeks until neurofilament reductions reached their nadir) and the time needed for that biological activity to translate to clinical benefit.

To mitigate potential risks of the relatively short and small study design, delayed-start analyses at later timepoints (e.g., Week 52) were prospectively planned via integration of data from Studies 101 and 102.

#### 2.6.1.4. Rationale for Key Pharmacodynamic and Efficacy Endpoints

Study 101 and the OLE incorporated a battery of measures to assess the effects of tofersen on the underlying disease biology, strength, function, QoL, and survival. These measures are described briefly below.

#### 2.6.1.4.1. ALSFRS-R

Change from baseline on ALSFRS-R total score was assessed as the primary efficacy endpoint for VALOR. This is a 12-item scale that assesses function in 4 domains: bulbar, fine motor, gross motor, and respiratory [Cedarbaum 1999]. Each item is rated on a scale of 0 to 4, thus generating an ALSFRS-R total of score of 0 (maximum disability) to 48 (no disability). ALSFRS-R has been correlated with QoL, caregiver burden, and survival [Gordon 2010; Gordon 2013; Kaufmann 2005; Lou 2010]. A minimally clinically important difference (MCID) threshold has not been established for this measure, at least in part because of its ordinal nature. As with other ordinal scales, change in ALSFRS-R scores typically follow a nonlinear, S-shaped curve, where a 1-point change is not consistent across the scale. However, it is well recognized that even a small drop in an individual's ALSFRS-R can significantly inhibit functional abilities. For example, a 1-point drop can reflect the difference between being able to walk with assistance to being nonambulatory. A 1-point drop has also been associated with a 7% increase in risk of death or tracheostomy [Kaufmann 2005].

ALSFRS-R has been commonly used as a primary endpoint to assess function in daily activities in ALS clinical trials [Mitsumoto 2014]. Guidelines on development of drugs to treat ALS published by the FDA [FDA 2019] and European Medicines Agency [EMA 2015] reference the ALSFRS-R as a suitable primary endpoint to assess effectiveness.

The measure has its strengths, including ease of use and correlation with survival. However, it also has limitations, which have the potential to interfere with evaluation of treatment effect, particularly in studies of relatively small size and short duration [Bakers 2021; Bedlack 2016; Fournier 2020; Franchignoni 2013; Proudfoot 2016; van Eijk 2021]. Importantly, the scale is associated with large intra-participant variability and in many people living with ALS, the decline is nonlinear (Figure 1) [Proudfoot 2016; Ramamoorthy 2022]. The subjectivity of the scale makes it susceptible to motivated scoring, e.g., to achieve a lower baseline score for trial eligibility. Furthermore, the scale lacks global standardized administration procedures. Originally developed in 1991, Cedarbaum et al. published a revision to the ALSFRS scale (referred to as ALSFRS-R) in 1999 intended to incorporate assessment of respiratory function [Cedarbaum 1999]. Groups have continued to adapt the scale, though these adaptations have not been published to date. An adaptation of the ALSFRS-R scale generated by Barrow Neurological Institute was used in Studies 101 and 102 (referred to as the "Global ALSFRS-R") for all participants except Japanese participants. This adaptation, which has been utilized across studies conducted in partnership with the Northeast Amyotrophic Lateral Sclerosis Consortium, includes updates to questions 4 (handwriting) and 11 (orthopnea) from the original publication to clarify answers and provide guidance on how to score these items. A Japanese translation of the original scale was used for all Japanese participants in Studies 101 and 102 (referred to as the "Japanese ALSFRS-R") [Cedarbaum 1999]. Japanese participants also completed 2 additional ALSFRS-R questions (questions 5a and 11) that had been modified in a Japanese adaptation of the ALSFRS-R (called the "Ohashi version") [Ohashi 2001].

#### 2.6.1.4.2. Total CSF SOD1

Given tofersen's mechanism of action, levels of total SOD1 protein in the CSF were measured to indirectly assess target engagement. Given its relevance, change in total SOD1 protein in the

CSF was evaluated as a key secondary endpoint (ranked first in order of hierarchical testing) in VALOR.

Notably, available assays including the immunoassay utilized in the tofersen development program (Covance Laboratories Inc., LLC) are not specific to toxic SOD1 protein and likely reflect a measure of primarily native SOD1 protein. In people living with SOD1-ALS, levels of total CSF SOD1 protein are stable over time and do not differ from those in normal individuals or those with broad ALS. Importantly, levels of total CSF SOD1 do not correlate with disease stage or progression as measured by baseline ALSFRS-R or monthly change of the ALSFRS-R, respectively [Winer 2013]. Therefore, a reduction in total CSF SOD1 is suitable as an indirect measure of target engagement but is not expected to predict clinical effect of tofersen. Though a target therapeutic threshold for total CSF SOD1 reduction has not been established, Biogen considered reductions of at least 20% as evidence of indirect target engagement because this level of reduction is greater than assay and biological variability.

#### 2.6.1.4.3. Plasma NfL

With the expectation that lowering of the causative protein (toxic SOD1 protein) would lead to reduced axonal injury and neurodegeneration, plasma NfL was evaluated as a key secondary endpoint (ranked second in order of hierarchical testing) in VALOR.

In VALOR and its OLE, human ethylenediaminetetraacetic acid plasma and CSF samples were analyzed to determine relative NfL concentrations using an immunoassay on the Siemens Atellica® IM. The Siemens NfL assay is a 2-site sandwich immunoassay using direct chemiluminometric technology. The Atellica is a fully automated random-access instrument that reduces sample-to-sample variability and minimizes plate bias.

#### 2.6.1.4.4. SVC

Vital capacity is a measure of respiratory function/strength that assesses the maximum amount of air expelled from the lungs after a maximum inhalation [Paganoni 2014]. The speed of the exhalation dictates whether the vital capacity is measured as forced vital capacity (FVC) or SVC. Though highly correlated, SVC (rather than FVC) was selected, as it is considered less variable in individuals with impaired breathing, spasticity, and/or significant bulbar dysfunction [Andrews 2018; Pinto and de Carvalho 2017; Sanjak 2010].

Change in SVC has been shown to be predictive of respiratory failure, tracheostomy, or death in individuals with ALS [Andrews 2018; Baumann 2010; Chiò 2009; Pinto and de Carvalho 2017; Traynor 2004]. Specifically, Pinto et al. found that a 1% decrease in percent-predicted values of SVC or FVC was associated with an increase in the probability of death by 1.02 [Pinto and de Carvalho 2017]. Andrews et al. used a Cox proportional hazards regression model to evaluate time to key events. They concluded that a slowing in the rate of SVC decline by 1.5 percent-predicted per month reduced the risk in first onset of respiratory insufficiency or death, first occurrence of tracheostomy or death, and death at any time after 6 months by 22%, 23%, and 23%, respectively ($p < 0.001$) [Andrews 2018].

Given its clinical importance, SVC was assessed as a key secondary endpoint (ranked third in order of hierarchical testing) in VALOR. To ensure quality of SVC data for eligibility assessment and efficacy analysis in the studies, SVC efforts were evaluated for acceptability and

repeatability in accordance with the criteria established by the American Thoracic Society (ATS) and the European Respiratory Society.

#### 2.6.1.4.5. HHD Megascore

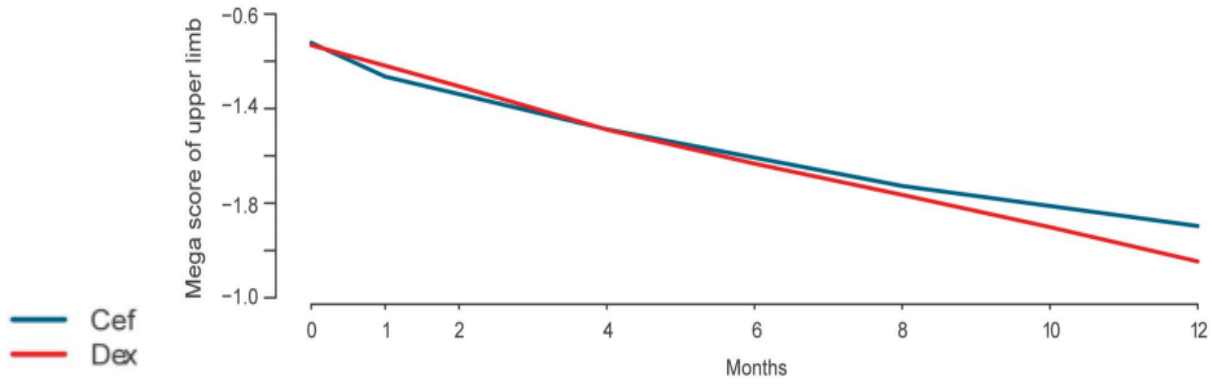
Loss of muscle strength is a hallmark of ALS as people living with ALS become progressively weaker over time, and strength loss is directly related to declining function in ALS [Cudkowicz 2013]. The weakness in ALS is relentlessly progressive; increases in strength are inconsistent with the natural history of the disease (Figure 2, Figure 3) [Cudkowicz 2013; Shefner 2016; Thakore 2021].

Quantitative muscle strength testing using HHD was incorporated as a key secondary endpoint in VALOR. The HHD megascore was calculated by averaging Z scores for 16 individual muscle groups in the upper and lower extremities (left and right shoulder flexion, elbow flexion, wrist extension, index finger abduction, thumb abduction, fifth digit abduction, knee extension, and ankle dorsiflexion).

While assessment of muscle strength is highly clinically relevant, the location, severity, and progression of weakness is highly variable among people living with ALS. The onset of weakness in ALS is often asymmetric, even focal. The composite HHD megascore is a single score across 16 muscles, which may limit the ability of the score to detect a treatment effect on focal/asymmetric weakness, especially over the short term.

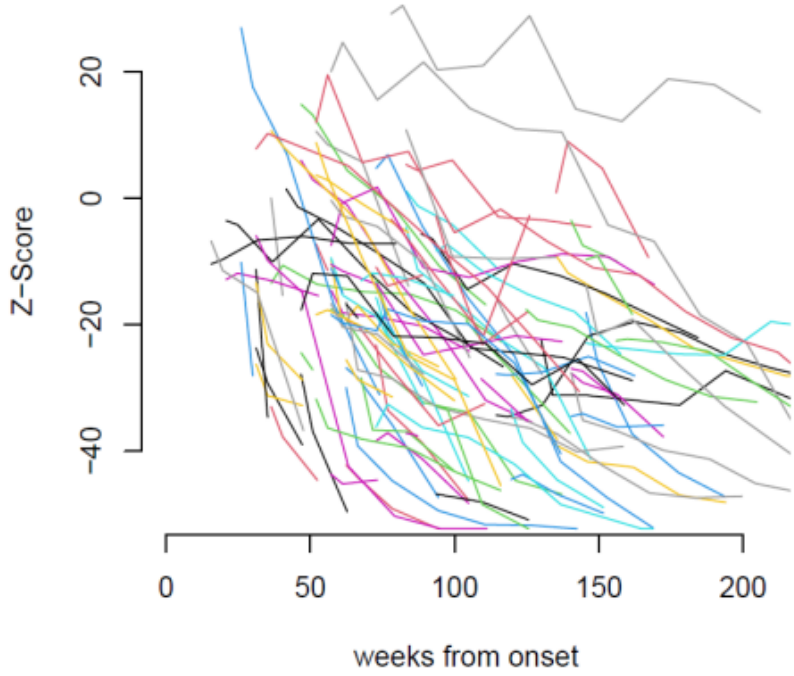
Strength loss is the direct result of motor neuron loss in SOD1-ALS, but it is estimated that clinically apparent strength loss is not discernable until approximately 50% to 80% of these neurons innervating a muscle or muscle group have degenerated [McComas 1971]. Strength is initially preserved because surviving motor neurons can sprout new terminal branches to reinnervate nearby muscle fibers that have lost connection to their original motor neuron [Bruneteau 2015; Fischer 2004]. Thus, weakness is apparent only after motor neuron loss exceeds the ability of surviving motor neurons to reinnervate muscle fibers. In the case of time-limited, moderate motor neuron loss, such as less severe polio, this ability of surviving motor neurons to reinnervate muscle fibers also provides a mechanism whereby strength may gradually recover [Halstead 1998]. Therefore, it is to be expected that recovery of strength would lag behind evidence of reduced axonal injury or neurodegeneration, as the sprouting of new nerve terminal branches and reinnervation of muscle fibers is not immediate.

**Figure 2: ALS Natural History Data: HHD Megascore in Dexpramipexole (Dex) and Ceftriaxone (Cef) Trials**




Source: from [Shefner 2016].

**Figure 3: ALS Natural History Data: Spaghetti Plots of HHD Z-Megascore (Total of 18 Muscle Groups, Arms and Legs) Trajectories for 100 Participants**




Source: from [Thakore 2021].

### 2.6.1.4.6. Survival

Recognizing the progressive and fatal nature of SOD1-ALS, time to death or PV and time to death were assessed as key secondary endpoints in VALOR. 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) [Benatar 2018a; Cudkowicz 2013] in an effort to differentiate between permanent ventilation and potential acute reversible illnesses (e.g., pneumonia) necessitating

temporary ventilatory support. Given variable standards of care for tracheostomy placement, mere tracheostomy was not considered an event, unless the threshold for ventilatory support was met.

While survival is considered the gold standard for demonstrating efficacy, it necessitates studies of adequate size and duration. Given the relatively short duration of VALOR, data from VALOR and OLE were integrated to enable comparison of survival with early- versus delayed-start tofersen over a longer period of time. To supplement these analyses, vital status data were retrospectively collected postwithdrawal for participants who withdrew from Study 101 or the OLE for reasons other than death.

Ventilation data and other relevant data (e.g., serious adverse events [SAEs], concomitant non-drug procedures and hospitalizations) were compiled and reviewed by an independent, central, blinded endpoint adjudication committee to determine whether and when VALOR participants reached the endpoint of PV or death in VALOR or the OLE.

#### 2.6.1.4.7. Quality of Life

Several QoL measures were included as exploratory endpoints in VALOR to evaluate the effects of tofersen on aspects of the disease not quantified with traditional ALS measures.

The ALSAQ-5 is a disease-specific participant-reported health status questionnaire intended for use in people living with ALS or other motor neuron diseases. The scale utilizes 5 questions to assess physical mobility, activities of daily living and independence, eating and drinking, communication, and emotional functioning [Gołąb-Janowska 2010]. Responses to each question range from 0 (“never”) to 4 (“always” or “cannot do at all”), with lower scores indicating better health status.

The EQ-5D-5L is a generic health status assessment that measures 5 dimensions, including mobility, self-care, usual activities, pain/discomfort, and anxiety/depression; higher scores on this 5-point scale are associated with better QoL. A summary utility index value was calculated for participants with non-missing data for all 5 questions at a visit. The “crosswalk” method (EuroQol Group) was used to map the EQ-5D-5L to the EuroQol 5 Dimension, 3 Level Questionnaire United Kingdom (EQ-5D-3L UK) value set, as the value sets for the EQ-5D-5L are still under development. The EuroQoL 5 Dimension - visual analog scale (EQ-5D-VAS) records the respondent’s self-rated health on a vertical scale ranging from 0 to 100, where 100 is considered “best imaginable health state” and 0 is considered “worst imaginable health state.” EQ-5D has shown that QoL decreases with increasing disease severity in people living with ALS [Kiebert 2001].

Fatigue is reported as one of the most bothersome and undertreated symptoms associated with ALS [Nicholson 2018]. The Fatigue Severity Scale (FSS) has been used to measure fatigue in a variety of conditions, including ALS [Krupp 1989; McElhiney 2009; Rabkin 2009; Ramirez 2008]. It is a 9-item self-reported questionnaire designed to assess fatigue across 3 domains, including life participation, sleep, and daily activities. Higher scores are indicative of greater fatigue in everyday life.

### 2.6.2. Statistical Methodology

Study 101 was completed in July 2021 with final database lock in August 2021; the OLE remains ongoing, with an interim data cutoff of the OLE taken in July 2021.

Given the relatively short duration of VALOR, the development program was prospectively designed to evaluate crossover in VALOR and its OLE by integrating outcomes across the studies. This integration of data from VALOR and OLE enables the comparison of early-start and delayed-start tofersen. These integrated analyses follow the ITT principle whereby all participants who underwent randomization in VALOR are included according to their original trial-group assignment, regardless of the rate of disease progression, adherence to study treatment, early termination of the trial, or crossover to the tofersen group. Analyses of VALOR and integrated efficacy and safety analyses based on the July 2021 data cutoff were conducted according to Final SAP V2.0 of VALOR, Final ISE SAP V2.0, and Final integrated summary of safety (ISS) SAP V2.0, respectively, all of which were finalized prior to the database lock for VALOR.

A second interim data cutoff of the OLE was performed on 16 January 2022, when all participants randomized in VALOR had the opportunity for at least 1 year of follow-up across studies. Ahead of locking the database (28 February 2022) and analyzing the data for the 16 January 2022 data cutoff, the ISE SAP V3.0 was completed. Biogen team members who worked on ISE SAP V3.0 were firewalled from ongoing OLE data.

Dates of VALOR and OLE data cutoffs and SAP revisions are summarized in Table 3.

**Table 3: Summary of VALOR and OLE Data Cutoffs and SAP Revisions**

| Date | Milestone/Activities |
|---|---|
| 09 June 2021 | Version 1.0 of VALOR ISE, and ISS SAPs finalized |
| 16 July 2021 | Last participant out of VALOR<br>Interim data cutoff 1 of the OLE |
| 14 August 2021 | Version 2.0 of VALOR, ISE, and ISS SAPs finalized |
| 16 August 2021 | Final database lock for VALOR<br>Interim database lock 1 for the OLE (16 July 2021 data cutoff) |
| 17 August 2021<br>to<br>28 February 2022 | • Initial readout of VALOR and ISE/ISS analyses.<br>• Only key personnel involved in preparation and interpretation were unblinded to individual data; this team remained firewalled from study conduct and ongoing data collection/cleaning activities. Site staff, study participants and vendors remained blinded to individual treatment assignments from VALOR. |
| 02 February 2022 | Version 3.0 of ISE and ISS SAPs finalized<br>o *Incorporated analyses in the full ITT population with adjustment for baseline (VALOR) plasma NfL as a covariate* |
| 28 February 2022 | Interim database lock 2 for the OLE (16 January 2022 data cutoff) |
| 17 August 2022 | Interim database lock for safety data pertaining to 120-day safety update (15 July 2022 data cutoff); based on ISS SAP V3.0 |

The primary analysis population in VALOR is FPS (mutation/slope). The JRT was used for the statistical inference of the primary endpoint of change from baseline to Week 28 in ALSFRS-R total score. The estimated between-group difference was obtained from the ANCOVA model for change from baseline to Week 28 in the ALSFRS-R total score in conjunction with multiple

imputation (MI) to handle missing data after withdrawal from the study. Key preplanned analyses in VALOR include the following:

- Sensitivity analysis in FPS (mutation/slope) and SPS (mutation/slope), respectively, with baseline plasma NfL as a covariate for the primary endpoint.
- Subgroup analyses in FPS (NF-based) and SPS (NF-based) for primary endpoint and secondary endpoints.
- Descriptive analyses in the ITT population for clinical function, biomarker, QoL, and time to event endpoints.
- Key prespecified ISE analyses in the ITT population based on the 16 January 2022 OLE data cutoff (ISE SAP V3.0) include all clinical function and QoL endpoints in the ITT population with adjustment for baseline plasma NfL as a continuous covariate in the ANCOVA + MI analysis instead of disease duration.
- Log-rank test (stratified by median baseline plasma NfL) and Cox proportional hazards model with adjustment for baseline plasma NfL for time to event endpoints.

Additional key modified/exploratory analyses incorporating learnings from the VALOR readout include the following:

- Incorporating baseline plasma NfL as a covariate instead of disease duration in the ANCOVA + MI model for the primary, secondary, and exploratory endpoints in the VALOR ITT population.
- Exploratory analyses evaluating an alternative method for implementing the JRT for both the VALOR ITT population change from baseline to Week 28 ALSFRS-R total score and the ISE analysis of change from baseline to Week 52 ALSFRS-R total score.
- Statistical modeling work to evaluate the relationship between early reduction in NfL due to tofersen treatment and later improvement in clinical outcome.

Further detail of the statistical methodology are provided in Appendix 1, Section 9.1 to Section 9.4.

# 3. DEMONSTRATION OF POTENTIAL SURROGACY OF NFL

## 3.1. Utility of Neurofilament in Neurodegenerative Diseases

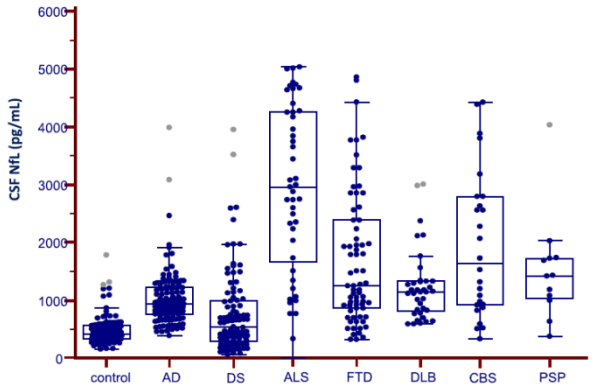
Neurofilaments are intermediate filaments that are uniquely expressed in neurons. Neurofilaments are heteropolymers composed of the light (NfL), middle (neurofilament medium chain), and heavy chains (neurofilament heavy chain), plus α-internexin in the CNS and peripherin in the PNS [Yuan 2017].

When axons are injured or degenerating, neurofilament leaks into the interstitial fluid. From there, it passes into the CSF and blood, where levels can be quantified [Gaetani 2019; Khalil 2018].

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. Neurofilament levels in ALS are among the highest across neurodegenerative diseases, with levels being 7- to 10-fold higher in people living with ALS than in neurologically healthy controls (Figure 4 and Figure 5) [Bridel 2019; Delaby 2020; Falzone 2020; Skillbäck 2017]. This is likely because ALS is characterized by a relatively rapid degeneration of motor neurons, where the longest axons in the body are found, containing relatively large quantities of neurofilament [Olsson 2019].

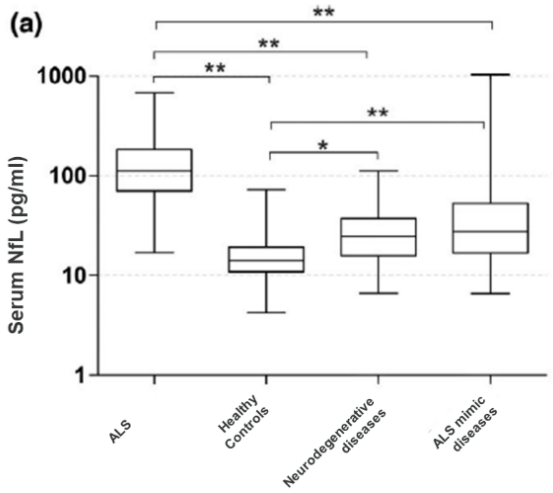
These elevations are observed in both the blood and CSF, with high correlations between matrices [Benatar 2018b; Gaiottino 2013; Lu 2015; Steinacker 2017].

**Figure 4: CSF Neurofilament Levels in People Living with ALS Compared to People with Other Neurodegenerative Diseases and Healthy Controls**




Box and whisker plots of the median concentrations of CSF NfL in control participants and people living with Alzheimer disease (AD), down syndrome (DS), dementia with Lewy bodies (DLB), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal syndrome (CBS), and progressive supranuclear palsy (PSP). The central black lines show the median values, and regions above and below these lines show the upper and lower quartiles, respectively. Outliers (indicated with grey circles) are defined as a value that is larger than the upper quartile plus 3 times the interquartile range.
Source: from [Delaby 2020].

**Figure 5: Serum Neurofilament Levels in People Living with ALS Compared to People with Other Neurodegenerative Diseases and Healthy Controls**




Boxes are median concentration and interquartile range. Whiskers are lowest and highest values. Levels are plotted on a 10-logarithmic scale.
Source: adapted from [Falzone 2022].

Detectable prior to clinical signs and symptoms, elevations in neurofilament continue to rise for approximately 6 to 20 months after clinical onset before stabilizing [Benatar 2018b; Benatar 2020; Brodovitch 2021; De Schaepdryver 2019b; Feneberg 2018; Gaiottino 2013; Gille 2019; Lu 2015; Poesen 2017; Thompson 2022]. Significant reductions in neurofilament levels are not expected over time in the absence of an intervention. Elevated and increasingly variable neurofilament levels have been observed with aging (as well as decreased brain volume loss and body mass index) in healthy controls [Khalil 2020; Manouchehrinia 2020; Vågberg 2015]. However, most studies of people living with ALS have not replicated these observations, likely because the magnitude of elevations resulting from axonal degeneration in ALS far exceeds the modest increases due to axonal loss and subclinical comorbidities associated with normal aging [Feneberg 2018; Rossi 2018; Thompson 2022; Verde 2019].

The ALS academic community has worked to characterize the behavior of neurofilament in ALS, particularly over the past decade. Based on these findings, there are multiple contexts of use for neurofilament in ALS clinical trials:

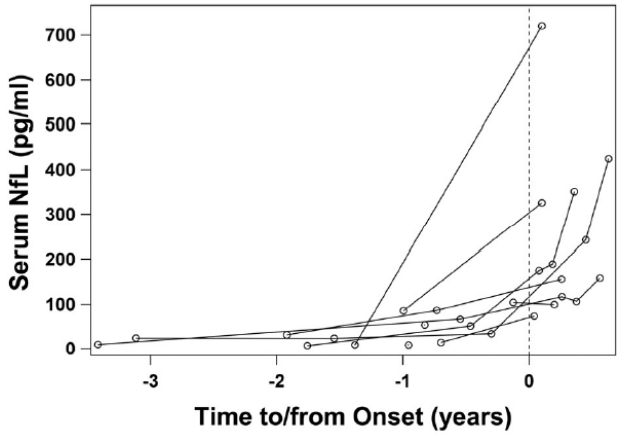
- As a susceptibility/risk biomarker to identify disease activity in presymptomatic at-risk carriers
- As a prognostic biomarker of disease progression and survival to control for disease heterogeneity at Baseline (e.g., incorporation of neurofilament as a covariate to adjust for chance imbalances between study treatment arms)
- As a biomarker of treatment response ± a surrogate biomarker reasonably likely to predict clinical benefit

### 3.1.1. Neurofilament as a Susceptibility/Risk Biomarker

Data from McComas et al. indicate that a considerable (> 70%) loss of motor neurons occurs before the onset of symptoms of weakness in people living with ALS [McComas 1971]. Consistently, elevations in neurofilament levels have been observed before emergence of clinically manifest ALS [Benatar 2019; De Schaepdryver 2019a]. In a longitudinal natural history study (the Presymptomatic Familial ALS or Pre-fALS Study) of presymptomatic at-risk carriers, elevated neurofilament levels were observed 6 to 12 months prior to detectable clinical signs or symptoms in participants with rapidly progressive *SOD1* mutations (Figure 6; [Benatar 2018b]). Similar observations were made in the broader ALS population (including those with and without confirmed genetic mutations), where retrospective sample testing showed elevations in serum pNfH levels increased during the presymptomatic period [De Schaepdryver 2019a].

Based on these observations, the tofersen ATLAS study is evaluating whether initiation of therapy in clinically presymptomatic *SOD1* mutation carriers with biomarker evidence of disease activity (elevated neurofilament levels) can halt or delay the onset of clinical signs and symptoms of the disease [Benatar 2022].

**Figure 6: Neurofilament Levels Pre- and Post-Phenoconversion in Causative Mutation Carriers**




Longitudinal changes in serum NfL concentration for phenoconverters. The x-axis shows years to or since the onset of symptoms or signs, which is marked by the vertical dashed line at year = 0.
Source: adapted from [Benatar 2018b].

### 3.1.2. Neurofilament as a Prognostic Biomarker of Disease Progression and Survival

Neurofilament levels correlate with disease progression rate and are prognostic for disease progression in ALS Figure 7 ()[Brodovitch 2021; De Schaepdryver 2020; Gille 2019]). Higher levels of neurofilament are associated with faster/greater decline on ALSFRS-R over time (Figure 8 ) [Gaiani 2017].

Neurofilament levels are also strongly prognostic of survival in ALS (

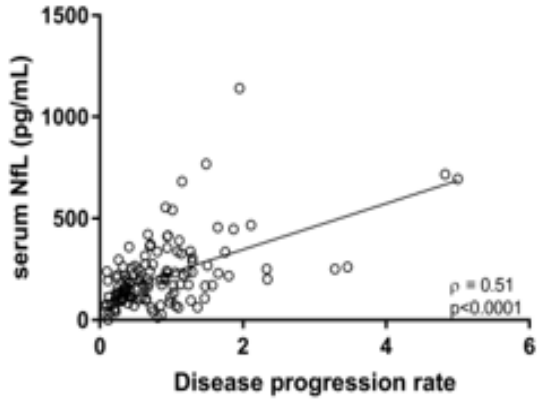
Figure 9 ) [Abu-Rumeileh 2020; Brettschneider 2006; De Schaepdryver 2020; Falzone 2022; Lu 2015; Rossi 2018; Thompson 2022; Thouvenot 2020; Vacchiano 2021; Zetterberg 2007]. A multicenter study found that plasma NfL levels were strongly associated with survival (hazard ratio [HR] for one SD increase in $\log_{10}$ plasma NfL 2.99, 95% confidence interval [CI]: 1.65 to

5.41; p = 0.016) and rate of disability progression, independent of other prognostic factors [Thompson 2022]. Importantly, plasma NfL was the only variable independently associated with shortened survival in Cox proportional hazards models incorporating clinical variables previously associated with survival (e.g., FVC, progression rate, time from symptom onset) and several blood analytes [Thompson 2022].

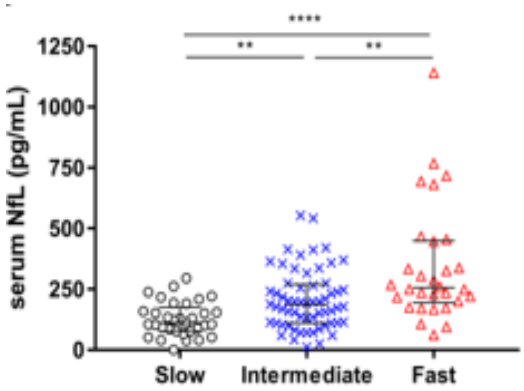
A consistent observation was made in the placebo-treated participants in VALOR, in which baseline neurofilament levels were more strongly correlated with longitudinal change in ALSFRS-R (Spearman correlation coefficient: -0.59; p = 0.0003) than with prerandomization ALSFRS-R slope decline (Spearman correlation coefficient: 0.32; p = 0.07) [Figure 10].

**Figure 7: Relationship Between Serum NfL and the Rate of Clinical Disease Progression**

**A**

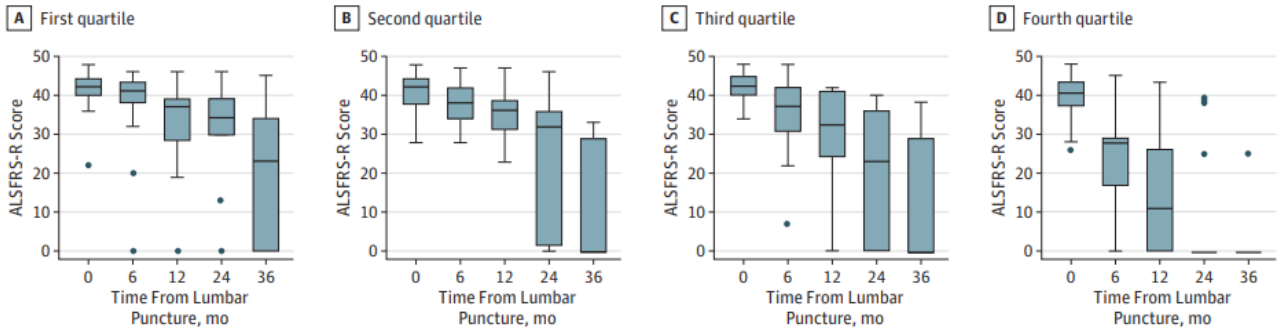



**B**




A: Positive correlation between the disease progression rate with the serum levels of NfL (Spearman's ρ = 0.51; p < 0.0001). B: Serum NfL levels in people living with ALS stratified according to their disease progression rate in slow (black circles), intermediate (blue crosses), and fast progressors (red triangles). Kruskal-Wallis test corrected for multiple comparison with the Dunn's post-hoc test.
Source: adapted from [Gille 2019].

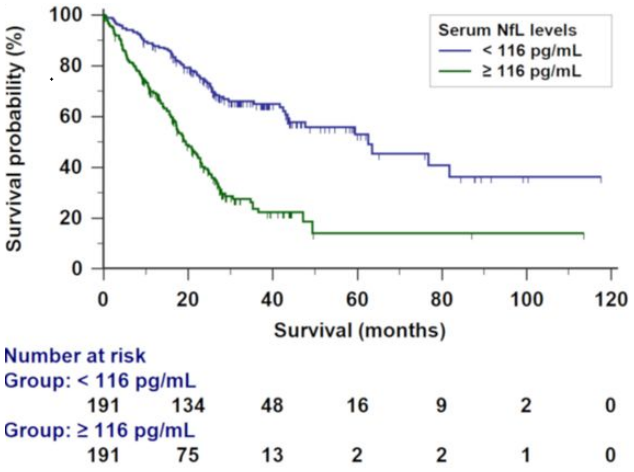
## Figure 8: Relationship Between Baseline Neurofilament Levels and Change on ALSFRS-R Over Time



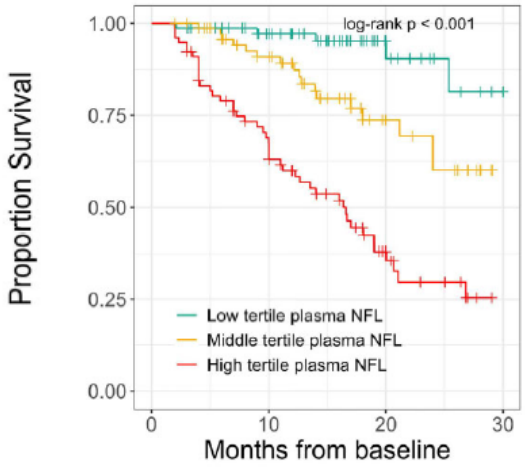

ALSFRS-R score change relative to time from lumbar puncture by log-transformed NfL quartiles. Error bars indicate SD.
Source: adapted from [Gaiani 2017].

## Figure 9: Correlation Between Neurofilament Levels and Survival

**A**

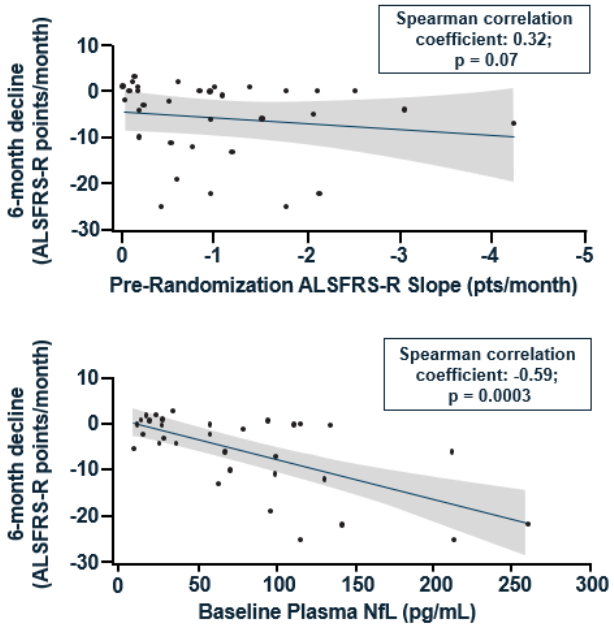



**B**




A: Kaplan-Meier survival estimates for people living with ALS classified into 2 groups based on the median for serum NfL levels.
Source: from [De Schaepdryver 2020].
B: Kaplan–Meier survival estimates for people living with ALS stratified by tertile of plasma NfL (n = 237) with log-rank test. p-value indicated for NfL tertiles.
Source: from [Thompson 2022].

**Figure 10: Baseline Plasma NfL and Prerandomization ALSFRS-R Decline as a Predictor of 6-Month Change in ALSFRS-R in VALOR Placebo Participants**

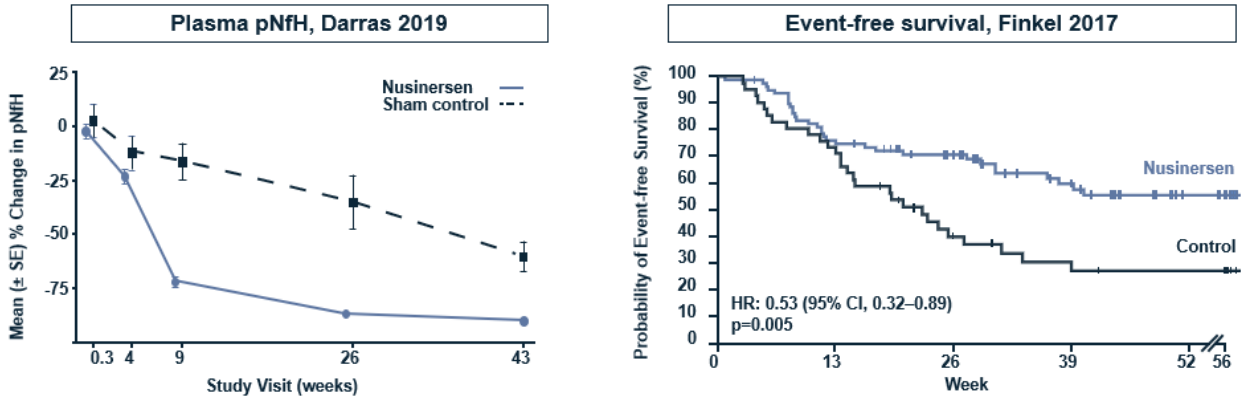



Baseline is defined as Day 1 value prior to the study drug. If Day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.

### 3.1.3. Neurofilament as a Marker of Treatment Response

In other neurological diseases including spinal muscular atrophy (SMA), neurofilament levels have been reduced in response to treatments associated with disease-modifying effects. These reductions have preceded discernable clinical benefit such as prolongation of event-free survival (Figure 11) [Finkel 2017].

**Figure 11: Change in pNfH and Probability of Event-Free Survival with Nusinersen Treatment Versus Sham-Control in Infantile Onset SMA**




SE: standard error.
Source: adapted from [Darras 2019; Finkel 2017]

In contrast, treatment-driven increases of neurofilament have been associated with a worsening of clinical function in C9orf72-ALS. In a Phase 1/2 study evaluating BIIB078 in adults with C9orf72-ALS, the ASO achieved robust target engagement, as evidenced by reductions in poly-GA and poly-GP levels in the CSF. However, at the top dose tested (90 mg) over 6 months, statistically significant increases in CSF NfL were observed relative to the placebo group. These increases in NfL were accompanied by trends of greater worsening across clinical outcome measures of function, strength, and quality of life [Van den Berg 2022].

Hypothesizing that reducing synthesis of SOD1 protein in SOD1-ALS would reduce axonal injury and neurodegeneration, neurofilament levels were evaluated in nonclinical and clinical studies of tofersen.

## 3.2. Tofersen Administration Was Associated with Reductions in NfL in Nonclinical and Clinical Studies

In G93A mutant mice, tofersen administration reduced neurofilament levels, preserved compound muscle action potential, maintained weight and motor performance, and prolonged survival [McCampbell 2018].

In the tofersen Phase 1 multiple-ascending-dose study (Study 101 Part B), reductions in neurofilament were observed in participants with SOD1-ALS who received tofersen 100 mg over 3 months. Plasma NfL concentrations decreased by 34% (percent change in geometric mean from baseline) in the tofersen 100 mg group compared to an increase of 12% in the placebo group (absolute difference of 46%; $p = 0.0176$) [Miller 2020]. Similar reductions were observed with tofersen 100 mg across isoforms (NfL and pNfH) and matrices (plasma and CSF).

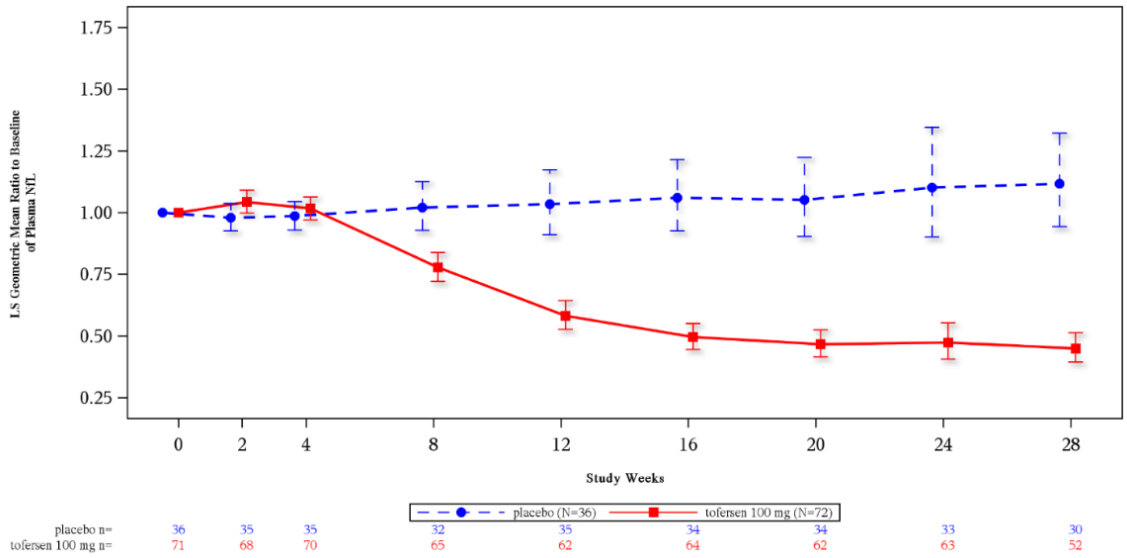
In VALOR and its OLE, tofersen-driven reductions in neurofilament were sustained over time and preceded discernable clinical benefit. Plasma NfL was formally tested as a key secondary endpoint in the FPS (mutation/slope) in VALOR. Descriptive analyses were performed in the SPS (mutation/slope) and ITT population. In the ITT population and these disease progression subgroups (FPS and SPS), robust reductions in neurofilament of 50% to 60% were observed (Table 4). Consistent with reductions observed in the FPS and SPS, plasma NfL levels were reduced by 55% (percent change in geometric mean from baseline) in the tofersen-treated participants, compared to a 12% increase in placebo-treated participants in the ITT population (absolute difference: 67%; post-hoc nominal $p < 0.0001$) in the ITT population (Table 4 and Figure 12). Reductions in neurofilament were observed in nearly all tofersen-treated participants in VALOR (Figure 13). In the integrated analyses of VALOR and its OLE, similar reductions in neurofilament were observed with delayed initiation of tofersen. Notably, reductions were similar across matrices (CSF and plasma) and analytes (NfL and pNfH) [Figure 14].

## Table 4: VALOR: Summary of Adjusted Geometric Mean Ratio to Baseline in Plasma NfL at Week 28

| Population | | Placebo | Tofersen |
|---|---|---|---|
| **ITT** | N<br>Adjusted GMR to baseline<br>Tof:plac ratio in GMR (95% CI)<br>Nominal p-value (ANCOVA+MI) | 36<br>1.12 | 72<br>0.45<br>0.40 (0.33, 0.49)<br><0.0001 |
| **FPS (Mutation/Slope)** | N<br>Adjusted GMR to baseline<br>Tof:plac ratio in GMR (95% CI)<br>Nominal p-value (ANCOVA+MI) | 21<br>1.20 | 39<br>0.40<br>0.33 (0.25, 0.45)<br><0.0001 |
| **SPS (Mutation/Slope)** | N<br>Adjusted GMR to baseline<br>Tof:plac ratio in GMR (95% CI)<br>Nominal p-value (ANCOVA + MI) | 15<br>0.95 | 33<br>0.50<br>0.52 (0.43, 0.63)<br><0.0001 |

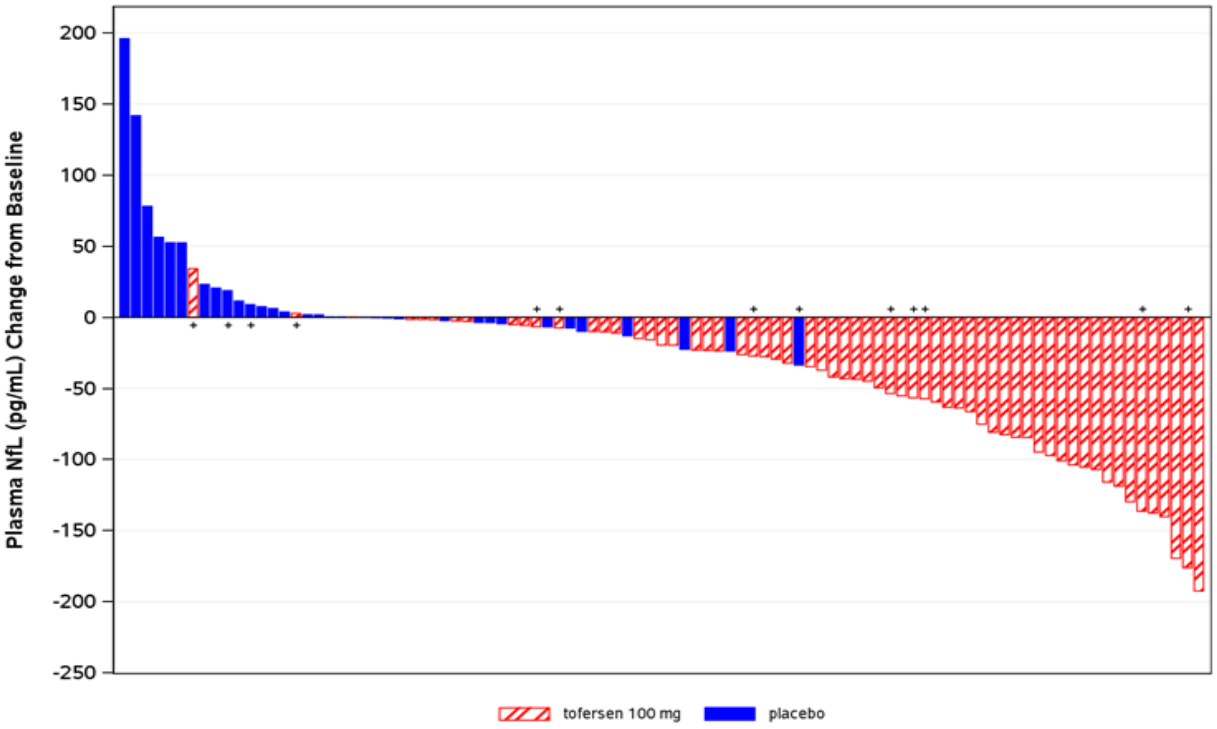
NOTE 1: Baseline is defined as day 1 value prior to the study drug. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Values below limit of quantitation (BLQ) are set to half of lower limit of quantitation (LLOQ, 4.9 pg/mL) in calculations.
NOTE 3: MI was used for missing data. Model included treatment, use of riluzole or edaravone, relevant baseline score and postbaseline values (natural log transformed data). Separate models for FPS (mutation/slope) and SPS (mutation/slope) were used and combined for ITT analyses.
NOTE 4: Adjusted geometric mean ratios to baseline, treatment differences in adjusted geometric mean ratios to baseline, and corresponding 95% CIs and nominal p-values were obtained from the ANCOVA model for change from baseline 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 analysis was based on natural log transformed data.

**Figure 12: VALOR: Plasma NfL Adjusted Geometric Mean Ratio to Baseline Values (95% CI) by Visit From ANCOVA Using MI - ITT Population**



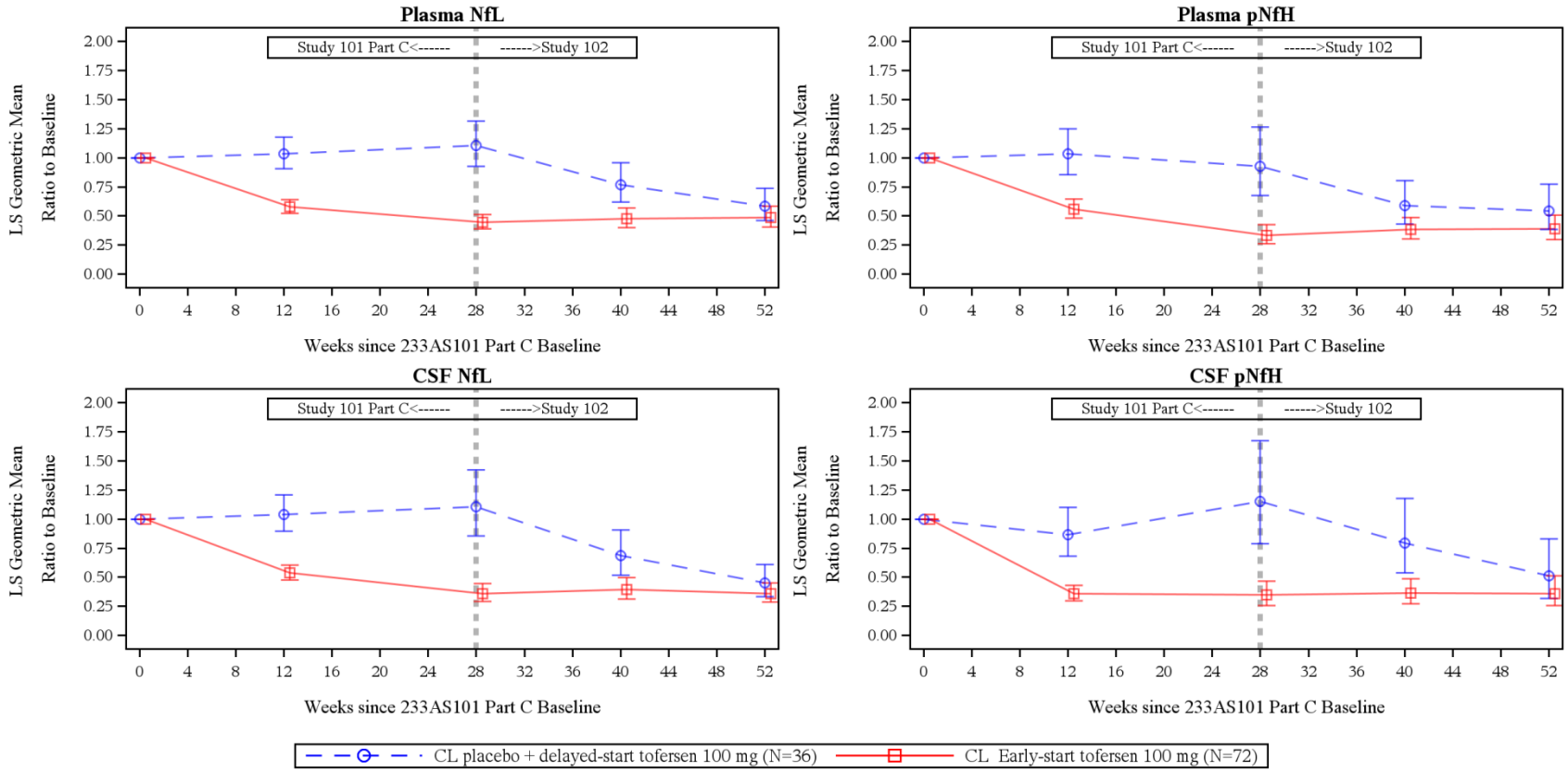

NOTE 1: Baseline is defined as day 1 value prior to the study drug. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Values BLQ are set to half of LLOQ (4.9 pg/mL) in calculations. Multiple 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 FPS (mutation/slope) and SPS (mutation/slope).
NOTE 4: The table at the bottom presents the number of participants with observed non-missing data at each visit.

**Figure 13: VALOR: Waterfall Plot of Plasma NfL Change From Baseline at Week 28 (Observed Data) - Study Completers; ITT Population**




NOTE 1: Observed data are presented for only study completers with a valid NfL result at Day 169 or Day 197.
NOTE 2: For completers with Day 197 data available, change from baseline at Day 197 is presented. For completers with missing Day 197 data, the Day 169 plasma NfL assessment is presented with +.
NOTE 3: Values below limit of quantitation (BLQ) are set to half of lower limit of quantitation (LLOQ, 4.9 pg/mL) in calculations.
Abbreviations: NfL = neurofilament light chain.

**Figure 14: VALOR and OLE ISE: Line Plot of Plasma NfL, CSF NfL, Plasma pNfH, CSF pNfH LS Geometric Mean Ratio to Baseline Values (95% CI) by Timepoint from ANCOVA 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 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.

## 3.3. Relationship Between NfL and Clinical Outcomes

### 3.3.1. Reductions in Neurofilament Preceded and Predicted Slowing of Clinical Decline

Tofersen-induced reductions in neurofilament reached their nadir by approximately Week 16, after SOD1 protein levels were reduced (Figure 12). These neurofilament reductions preceded discernable slowing of decline in strength, function, and QoL and reduction of risk of death-equivalent events, which were not clearly demonstrated until Week 52 and beyond.

Figure 13 illustrates reductions in neurofilament observed in nearly all tofersen-treated participants in VALOR. When considering the relationship between treatment-driven lowering of neurofilament and slowing of clinical decline, one must account for differing rates of natural disease progression across study participants (e.g., participants with higher baseline neurofilament levels are expected to lose more function over the study period than those with lower baseline levels). In aggregate analyses, this is done by incorporating baseline plasma NfL level as a covariate. After the VALOR readout in recognition of the importance of neurofilament reductions, a statistical model with a causal inference component was developed to formally interrogate the relationship on an individual basis.

This model assesses the relationship between early tofersen-drive reductions in plasma NfL at Week 16 and slowing of clinical progression over time. To do this, the model constructs a matched control for each of the tofersen-treated participants, using a causal inference framework informed by data from VALOR and its OLE from delayed-start participants. In doing so, the model is able to account for differing rates of natural disease progression, recognizing that those with higher baseline neurofilament levels are expected to lose more function than those with lower baseline levels.

The model deconstructs the observed treatment effect for a tofersen-treated participant into 3 components (Figure 15):

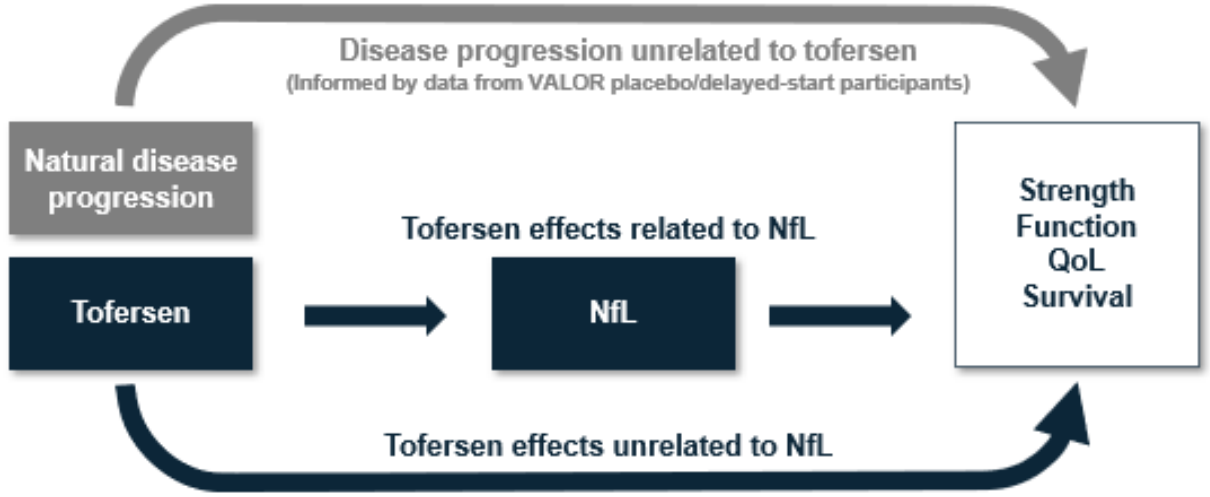
1. **Change due to natural disease progression**

   Estimated using data from VALOR placebo/delayed-start participants as follows:

   a. For analysis of measures of function/strength/QoL at Week 28, data from VALOR placebo participants were used to estimate the decline driven by natural disease progression.
   b. For analysis of survival endpoints over time, data from the delayed-start participants were used to conservatively estimate the event risk driven by natural disease progression.

2. **Change due to tofersen effect through the NfL pathway**
3. **Change due to tofersen effect through non-biomarker pathway/factors**

**Figure 15: Statistical Model Framework for Clinical Function**




More specifically, the model takes the baseline NfL level in a tofersen-treated participant to estimate what their NfL would have been at Week 16 without tofersen. It then uses those values to predict what would have occurred without tofersen at Week 28 for measures of strength, function, and QoL and over time for measures of survival. This enables comparison of the observed trajectory (with tofersen treatment) and the projected trajectory without treatment to estimate the magnitude of slowing in disease progression or reduction in risk associated with tofersen-driven reductions in plasma NfL.

The limitations of this model are recognized as it is solely based on data from VALOR and its OLE. To our knowledge, tofersen is the first therapy to demonstrate clear reductions in neurofilament in SOD1-ALS or in ALS more broadly. As such, no data from other therapies exist to replicate and support the framework of this model. That said, the results of the model reflect what would be expected with a therapy targeting the underlying pathophysiology of SOD1-ALS and are consistent with observations in VALOR and its OLE more broadly.

#### 3.3.1.1. Relationship Between NfL Lowering and Slowing of Decline in Strength, Function, and QoL

The model identifies a clear relationship between tofersen-driven reductions in plasma NfL at Week 16 and slowing of decline in function (ALSFRS-R, SVC), strength (HHD), and QoL (ALSAQ5, EQ-5D-5L) [Table 5].

For example, in a participant with a baseline plasma NfL consistent with the sample mean (at the 96.78 pg/mL), the model shows that every 10 pg/mL reduction in plasma NfL with tofersen administration at Week 16 is associated with a reduction in worsening on measures of clinical function, respiratory function, strength, and QoL at Week 28 (Table 5). This relationship is dynamic such that the difference would be greater in an individual with a higher baseline NfL level and faster disease progression where there would be greater opportunity to differentiate from natural disease progression.

The statistical model built using data from VALOR and its OLE supports that early (Week 16) tofersen-driven reductions in plasma NfL are directly associated with a reduction in worsening of clinical function (at Week 28) over time.

**Table 5: Relationship Between Tofersen-Driven Reductions in Plasma NfL at Week 16 and Slowing of Decline in Function, Strength, and QoL at Week 28**

| Clinical Outcome Measure | Reduction in Worsening with Tofersen (Versus Untreated) Per 10 Unit of NfL Lowering [a] |
|---|---|
| **ALSFRS-R Total Score** | 0.772 (p = 0.0038) |
| **Percent-Predicted SVC** | 1.451 (p = 0.0706) |
| **HHD Overall Megascore** | 0.029 (p = 0.1303) |
| **ALSAQ-5 Total Score** | 2.194 (p = 0.0056) |
| **EQ-5D-5L Utility Score** | 0.017 (p = 0.0894) |

[a] Reflects relationship at sample mean baseline plasma NfL (96.78 pg/mL).

### 3.3.1.2. Relationship Between NfL Lowering and Event-Free Survival/Overall Survival

The model also identifies a clear relationship between tofersen-driven reductions in plasma NfL at Week 16 and event-free survival/overall survival (Table 6).

For a participant with a baseline plasma NfL consistent with the sample mean (at the 96.78 pg/mL for the ITT population), the model shows the percent reduction in event risk associated with a 10 pg/mL reduction in plasma NfL ranges from 16.1% to 24.9% across the 4 survival endpoints. Though the number of events observed to date is limited, the overall finding that neurofilament reduction is associated with reduction in event risk is consistent across endpoints.

The statistical model supports that early (Week 16) tofersen-driven reductions in plasma NfL are directly associated with prolonged event-free survival/overall survival over time.

**Table 6: Relationship Between Reduction in Plasma NfL and Reduction in Event Risk Due to Tofersen Treatment.**

| Survival Endpoints | Tofersen Effect Through Biomarker | Percent Reduction In Event Risk With Tofersen Relative To 10 Unit Of NfL Lowering [a] |
|---|---|---|
| **Time To Death** | 0.0175 (p = 0.3690) | 16.1% |
| **Time To Death Or PV** | 0.0224 (p = 0.1119) | 20.1% |
| **Time To Death, PV, Or Withdrawal Due To Disease Progression** | 0.0287 (p = 0.0010) | 24.9% |
| **Time To Death With Additional Vital Status** | 0.0284 (p = 0.0318) | 24.7% |

[a] Reflects relationship at sample mean baseline plasma NfL (96.78 pg/mL).

### 3.3.2. Reductions of Neurofilament Are Reasonably Likely to Predict Clinical Benefit

In SOD1-ALS, production of toxic SOD1 protein leads to axonal injury/ degeneration and death of motor neurons. When these axons are injured or degenerating, neurofilament leaks into the interstitial fluid before passing into the CSF and blood where levels can be quantified. The ALS literature consistently demonstrates that neurofilament levels are prognostic for disease progression and survival, with higher levels associated with faster-progression and shorter survival. While reductions in neurofilament may not be observed with all ALS therapies (e.g., due to different mechanisms of action), a lowering of neurofilament is generally thought to represent a slowing of axonal injury and neurodegeneration, thus providing objective evidence of treatment effect.

Consistent with its mechanism, which targets the underlying and upstream cause of SOD1-ALS, tofersen administration led to reductions in neurofilament that were sustained over time. These reductions, which appeared to be maximized by approximately 16 weeks, preceded evidence of effects on clinical function, respiratory function, strength, quality of life, and survival, which were not clearly discernable until Week 52 and beyond.

Furthermore, the statistical model built using data from VALOR and its OLE supports that early (Week 16) tofersen-driven reductions in plasma NfL are directly associated with a reduction in worsening of clinical function (at Week 28) and prolonged event-free survival/overall survival over time.

Taken together, data from the literature and the tofersen program support that treatment-driven reductions in neurofilament are reasonably likely to predict clinical benefit in SOD1-ALS.

# 4. EFFECT OF TOFERSEN ON CLINICAL OUTCOME MEASURES

The observed difference on the VALOR primary analysis of change in ALSFRS-R score in the FPS (mutation/slope) did not reach statistical significance, thus making testing of secondary endpoints exploratory in nature. In the VALOR/OLE integrated analyses, no formal statistical testing of endpoints was performed. Nevertheless, the consistency of results favoring tofersen/early-start tofersen in VALOR and the integrated VALOR/OLE analysis across endpoints, populations, and analytical approaches is clear. The probability of these observations of clinical benefit being due to chance is extremely low given the strong concordance of these results. These results are described herein to provide clinical context for the position that reductions in neurofilament are reasonably likely to predict clinical benefit in SOD1-ALS.

As detailed in Section 2.6.2, descriptive analyses in the full ITT population were prespecified in the VALOR and ISE SAPs. However, analyses in ITT population with adjustment for baseline plasma NfL as a covariate was incorporated post-hoc based on learnings from VALOR and ALS literature. Forest plots have been included for each measure to illustrate the impact of adjusting for different covariates in different populations (ITT vs. disease progression subgroups defined by mutations/slope vs. disease progression subgroups defined by baseline plasma NfL levels).

## 4.1. Demographics and Baseline Characteristics

VALOR enrolled adults with weakness attributable to ALS and a confirmed *SOD1* mutation. In total, 108 participants were enrolled over 21 months at sites in Belgium, Canada, Denmark, France, Germany, Italy, Japan, the UK, and the US. A total of 42 unique *SOD1* mutations were included in the study, with the most common being p.Ile114Thr (n = 20), p.Ala5Val (n = 17), p.Gly94Cys (n = 6), and p.His47Arg (n = 5). 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 ITT population, approximately 62% of participants were receiving riluzole and 8% were receiving edaravone at Baseline (Table 7).

Baseline and disease characteristics were balanced across treatment arms for use of riluzole and/or edaravone and for characteristics reflective of the stage of disease, including time from symptom onset, total ALSFRS-R score, and percent-predicted SVC. However, baseline plasma NfL levels were approximately 15% to 25% higher in the tofersen group than in the placebo group. Consistently, the rate of decline on ALSFRS-R from Screening to Day 15 (an approximately 42-day period prior to achievement of steady state of tofersen) was greater in the tofersen group than in the placebo group. These imbalances, which were most apparent in the primary analysis population, suggest the tofersen group was progressing more quickly at Baseline than the placebo group. Subgroups defined according to baseline plasma NfL levels were more balanced.

## Table 7: Key VALOR Baseline Disease Characteristics

| | FPS | | | | SPS | | | | ITT | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mutation/slope | | NF-based | | Mutation/slope | | NF-based | | | |
| | **Placebo (n = 21)** | **Tofersen (n = 39)** | **Placebo (n = 16)** | **Tofersen (n = 38)** | **Placebo (n = 15)** | **Tofersen (n = 33)** | **Placebo (n = 20)** | **Tofersen (n = 34)** | **Placebo (n = 36)** | **Tofersen (n = 72)** |
| **Time from symptom onset** (months) | | | | | | | | | | |
| Median | 8.28 | 8.25 | 10.25 | 8.94 | 39.56 | 35.48 | 31.72 | 28.85 | 14.59 | 11.37 |
| Range | 2.4, 21.3 | 1.7, 18.5 | 2.4, 30.3 | 2.3, 59.9 | 11.8, 103.2 | 3.9, 145.7 | 3.0, 103.2 | 1.7, 145.7 | 2.4, 103.2 | 1.7, 145.7 |
| **Riluzole use** | | | | | | | | | | |
| n (%) | 13 (61.9) | 25 (64.1) | 11 (68.8) | 21 (55.3) | 9 (60.0) | 20 (60.6) | 11 (55.0) | 24 (70.6) | 22 (61.1) | 45 (62.5) |
| **Edaravone use** | | | | | | | | | | |
| n (%) | 1 (4.8) | 2 (5.1) | 1 (6.3) | 2 (5.3) | 2 (13.3) | 4 (12.1) | 2 (10.0) | 4 (11.8) | 3 (8.3) | 6 (8.3) |
| **Total CSF SOD1 protein** (ng/mL) | | | | | | | | | | |
| Mean (SD) | 118.1 (63.09) | 117.2 (62.04) | 108.6 (46.41) | 119.3 (58.30) | 135.8 (79.79) | 120.4 (49.73) | 139.0 (83.02) | 118.0 (54.76) | 125.5 (70.0) | 118.7 (56.28) |
| **Plasma NfL** (pg/mL) | | | | | | | | | | |
| Mean (SD) | 127.3 (94.40) | 146.2 (82.63) | 160.3 (85.51) | 159.4 (73.69) | 37.0 (29.51) | 47.6 (41.80) | 33.1 (20.91) | 36.2 (21.93) | 89.7 (86.47) | 100.4 (82.83) |
| Range | 9, 370 | 12, 329 | 78, 370 | 78, 329 | 8, 99 | 5, 211 | 8, 70 | 5, 74 | 8, 370 | 5, 329 |
| **Percent-predicted SVC** | | | | | | | | | | |
| Mean (SD) | 83.73 (17.87) | 80.30 (14.22) | 81.76 (19.6) | 82.61 (17.16) | 87.09 (14.82) | 84.20 (19.02) | 87.83 (13.52) | 81.51 (16.16) | 85.13 (16.53) | 82.09 (16.59) |
| **ALSFRS-R score** | | | | | | | | | | |
| Mean (SD) | 35.4 (5.7) | 36.0 (6.4) | 34.5 (5.8) | 36.4 (6.6) | 39.9 (5.1) | 38.1 (5.1) | 39.6 (4.9) | 37.5 (5.1) | 37.3 (5.81) | 36.9 (5.9) |
| **ALSFRS-R prerandomization slope** | | | | | | | | | | |
| Median | -1.512 | -1.340 | -1.440 | -1.173 | -0.165 | -0.300 | -0.208 | -0.314 | -0.892 | -0.747 |
| Range | -4.91, -0.42 | -8.30, -0.39 | -4.91, -0.42 | -8.30, -0.17 | -0.84, -0.02 | -0.77, 0.00 | -3.04, -0.02 | -6.60, 0.00 | -4.91, -0.02 | -8.30, 0.00 |
| **ALSFRS-R run-in slope decline** | | | | | | | | | | |
| Mean (SD) | -1.3 (3.91) | -1.8 (2.47) | -1.8 (2.71) | -1.7 (2.28) | 0.1 (1.87) | -0.1 (1.34) | 0.1 (3.48) | -0.3 (1.82) | -0.7 (3.25) | -1.0 (2.19) |

NOTE 1: ALSFRS-R run-in slope decline is based on the slope between Screening and postbaseline Day 15.
NOTE 2: All participants who received edaravone also received riluzole.
FPS: Faster-progression subgroup; SPS: Slower-progression subgroup

## 4.2. ALSFRS-R Total Score

### VALOR Primary Efficacy Analysis

ALSFRS-R change from baseline to Week 28 was assessed in the FPS (mutation/slope) as the primary endpoint in VALOR. No statistically significant difference was observed on the primary analysis of ALSFRS-R change from baseline to Week 28 in the FPS (mutation/slope) [difference: 1.2 points favoring tofersen; 95% CI: –3.2 to 5.5; p = 0.97 via JRT + MI analysis]. Statistical inference based on the ANCOVA + MI sensitivity analysis was consistent in showing no statistically significant difference (nominal p = 0.5998).

The difference between groups was larger (3.9-point difference; 95% CI: -1.00 to 8.86) when analyzed in the prespecified faster-progressing subgroup defined according to baseline plasma NfL levels in which baseline characteristics were more balanced (Figure 25).

### VALOR (Week 28) and VALOR/OLE (Week 52) Analyses in the ITT Population Adjusting for Baseline NfL

Over 28 weeks in the VALOR ITT population (n = 108), tofersen-treated participants experienced less decline on ALSFRS-R those on placebo (2.1-point difference; nominal p = 0.0904) [Table 8].

Over 52 weeks, the early-start group experienced less decline than the placebo/delayed-start group (3.5-point difference; nominal p = 0.0272), despite the opportunity for crossover to active tofersen at Week 28 (Table 8; Figure 16). Differences favoring early-start tofersen were observed across all 4 subdomains (Table 9).

As illustrated in Figure 17, trends consistently favored early-start tofersen in the ITT population and disease progression subgroups (mutation/slope and NF-based), with adjustment for different covariates. Specifically, the top row of the Forest Plot depicts the 52-week analysis described above in the ITT population with adjustment for baseline plasma NfL as a covariate. Recognizing that this analysis was incorporated in the ISE SAP only after the original VALOR readout, the following rows show the effects in the ITT population, FPS/SPS (mutation/slope), and FPS/SPS (NF-based), with different combinations of covariates. This plot reinforces the relative strength of different approaches to controlling for disease heterogeneity, with reduced variability when baseline NfL was incorporated as a covariate in the full ITT population; however, the effects favor early-start tofersen across analyses.

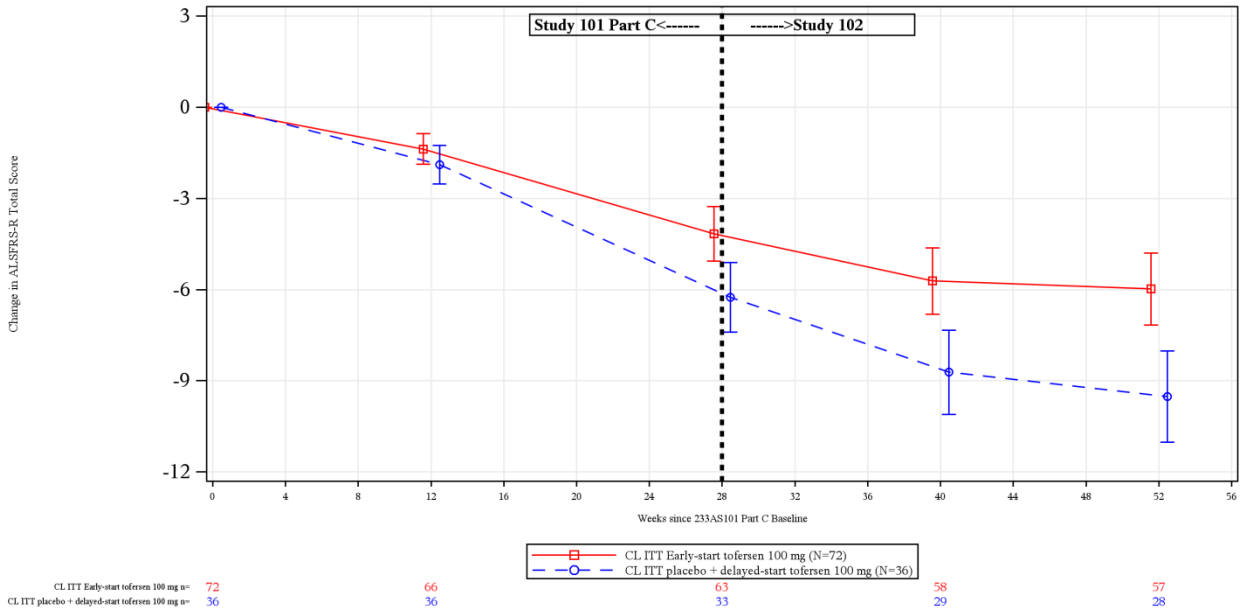
As described in Section 2.6.1.4.1, an MCID threshold has not been established for this measure at least in part due to the ordinal nature of the scale; however, each point lost represents the loss of important function. For example, a 1-point drop can reflect the difference between being able to walk with assistance to being nonambulatory. A 1-point drop has also been associated with a 7% increase in risk of death or tracheostomy [Kaufmann 2005].

## Table 8: Change in ALSFRS-R Total Score from VALOR Baseline (ITT Population)

| Endpoint | VALOR<br><br>Tofersen ( n =72) vs. Placebo (n = 36)<br><br>Change from Baseline to Week 28 | VALOR and OLE<br><br>Early-start tofersen (n = 72) vs. Placebo/Delayed-Start tofersen (n = 36)<br>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. Modified analyses are presented for VALOR; analysis for VALOR and OLE is based on January 2022 data cutoff and was prespecified in ISE SAP V 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 postbaseline 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. 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 16: Adjusted Mean Change (± SE) in ALSFRS-R Total Score from VALOR Baseline to Week 52 (VALOR + OLE; ITT Population)**



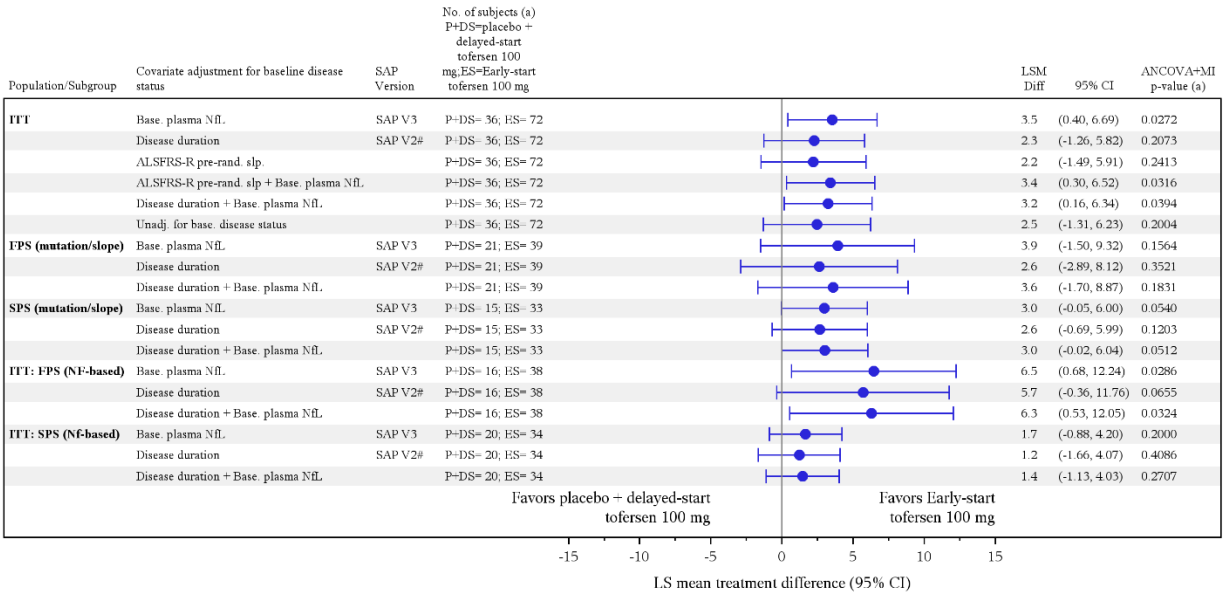

NOTE 1: Baseline is defined as day 1 value prior to the study drug and presented as Day 1. If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value.
NOTE 2: Multiple imputation including treatment group, use 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 participants, the Global ALSFRS-R is used. For Japanese participants, the Japanese (Ohashi) ALSFRS-R is used except for Question 5a and Question 11 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.

## Table 9: Change in ALSFRS-R Subdomain Scores from VALOR Baseline to Week 52 (ITT Population)

| Endpoint | Early-start tofersen (n = 72) | Placebo/delayed-start tofersen (n = 36) |
|---|---|---|
| **Gross Motor Skills Subdomain**<br>Adjusted mean<br>Tofersen-placebo: adjusted mean difference (95% CI)<br>p-value (ANCOVA+MI) | -1.1 | -2.1<br>1.0 (0.1, 1.8)<br>0.0227 |
| **Fine Motor Skills Subdomain**<br>Adjusted mean<br>Tofersen-placebo: adjusted mean difference (95% CI)<br>p-value (ANCOVA+MI) | -1.6 | -2.6<br>1.0 (0.1, 2.0)<br>0.0374 |
| **Bulbar Subdomain**<br>Adjusted mean<br>Tofersen-placebo: adjusted mean difference (95% CI)<br>p-value (ANCOVA+MI) | -1.4 | -1.7<br>0.3 (-0.7, 1.3)<br>0.5889 |
| **Respiratory Subdomain**<br>Adjusted mean<br>Tofersen-placebo: adjusted mean difference (95% CI)<br>p-value (ANCOVA+MI) | -1.9 | -3.1<br>1.2 (-0.1, 2.6)<br>0.0623 |

NOTE 1: Lower scores depict a worsening in function. Analysis for VALOR and OLE is based on January 2022 data cutoff and was prespecified. All p-values are nominal.
NOTE 2: The MI 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 postbaseline 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. 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.
NOTE 4: For non-Japanese participants, the Global ALSFRS-R is used. For Japanese participants, the Japanese (Ohashi) ALSFRS-R is used except for Question 5a and Question 11 where the Japanese translated Global ALSFRS-R is used. Four domain scores include bulbar function (Question 1 - Question 3), fine motor skills (Question 4 - Question 6), gross motor skills (Question 7 - Question 9), and respiratory function (Question 10 - Question 12). A positive change indicates an improvement.

## Figure 17: Forest Plot of ALSFRS-R Total Score Change from VALOR Baseline to Week 52 (VALOR + OLE)




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 the OLE based on 16 July 2021 data cutoff; 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 after 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 the OLE based on 16 January 2022 data cutoff.
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 FPS (mutation/slope) and SPS (mutation/slope) 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 FPS (mutation/slope) and SPS (mutation/slope) 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.
NOTE 4: FPS and SPS (mutation/slope) are disease progression subgroups based on mutation type and prerandomization slope as defined in the protocol.
NOTE 5: FPS and SPS (NF-based) are disease progression subgroups defined by the median baseline plasma NfL (>= and < Median (75.60 pg/mL)).
# No statistical testing was prespecified in SAP V2.0; all p-values are post-hoc for SAP V2.0 analyses.
P+DS = placebo + delayed-start tofersen 100 mg; ES = Early-start tofersen 100 mg.

## 4.3. Percent-Predicted SVC

### VALOR Key Secondary Efficacy Analysis

Change from baseline to Week 28 in percent-predicted SVC was assessed in the FPS (mutation/slope) as a key secondary endpoint in VALOR. In this subgroup, a difference of 7.9 percent-predicted favoring tofersen was observed (95% CI: -3.5 to 19.3); nominal p = 0.323 (JRT+MI).

The difference between groups was larger (9.91 percent-predicted difference (95% CI: -2.27 to 22.09) when analyzed in the prespecified faster-progressing subgroup defined according to baseline plasma NfL levels in which baseline characteristics were more balanced (Figure 26).

### VALOR (Week 28) and VALOR/OLE (Week 52) Analyses in the ITT Population Adjusting for Baseline NfL

Over 28 weeks in the VALOR ITT population (n = 108), tofersen-treated participants experienced less decline on SVC than those on placebo (8.5 percent-predicted difference; nominal p = 0.0128) [Table 10].

Over 52 weeks, the early-start group experienced less decline than the placebo/delayed-start group (9.2 percent-predicted difference; nominal p = 0.0159) [Table 10; Figure 18]. Over this 52-week period, 21.0% of the early-start participants and 12.8% of the delayed-start participants experienced an increase in percent-predicted SVC. Improvement in respiratory strength/function is inconsistent with the progressive natural history of the disease. In the dexpramipexole EMPOWER study, 47 of 942 participants (approximately 5%) experienced improvement in SVC over a similar time frame.

Figure 19 illustrates the consistent trends favoring early-start tofersen in the ITT population and disease progression subgroups (mutation/slope and NF-based), with adjustment for different covariates.

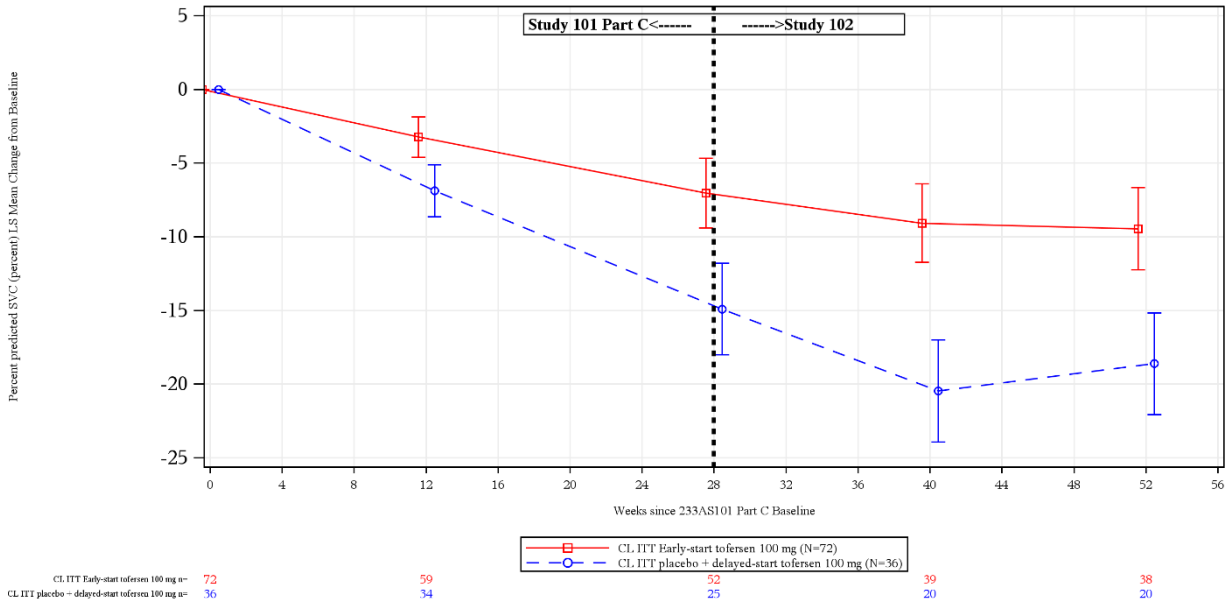
As detailed in Section 2.6.1.4.4, Andrews et al. found that a slowing in the rate of SVC decline by 1.5 percent-predicted per month reduced the risk in first onset of respiratory insufficiency or death, first occurrence of tracheostomy or death, and death at any time after 6 months by 22%, 23%, and 23%, respectively ($p < 0.001$) [Andrews 2018]. Therefore, the 8.5- and 9.2-percent-predicted differences observed between treatment groups at 28 and 52 weeks, respectively, are considered highly clinically relevant.

## Table 10: Change in Percent-Predicted SVC from VALOR Baseline (ITT Population)

| Endpoint | VALOR<br><br>Tofersen (n = 72) vs. Placebo (n = 36)<br><br>Change from Baseline to Week 28 | VALOR and OLE<br><br>Early-start Tofersen (n = 72) vs. Placebo/Delayed-start Tofersen (n = 36)<br><br>Change from Baseline to Week 52 |
|---|---|---|
| **Change from baseline in percent-predicted SVC** | | |
| Adjusted means: Tof, Placebo | -7.3, -15.8 | -9.4, -18.6 |
| Tofersen-placebo: adjusted mean difference (95% CI) | 8.5 (1.8, 15.2) | 9.2 (1.7, 16.6) |
| p-value (ANCOVA+MI) | 0.0128 | 0.0159 |

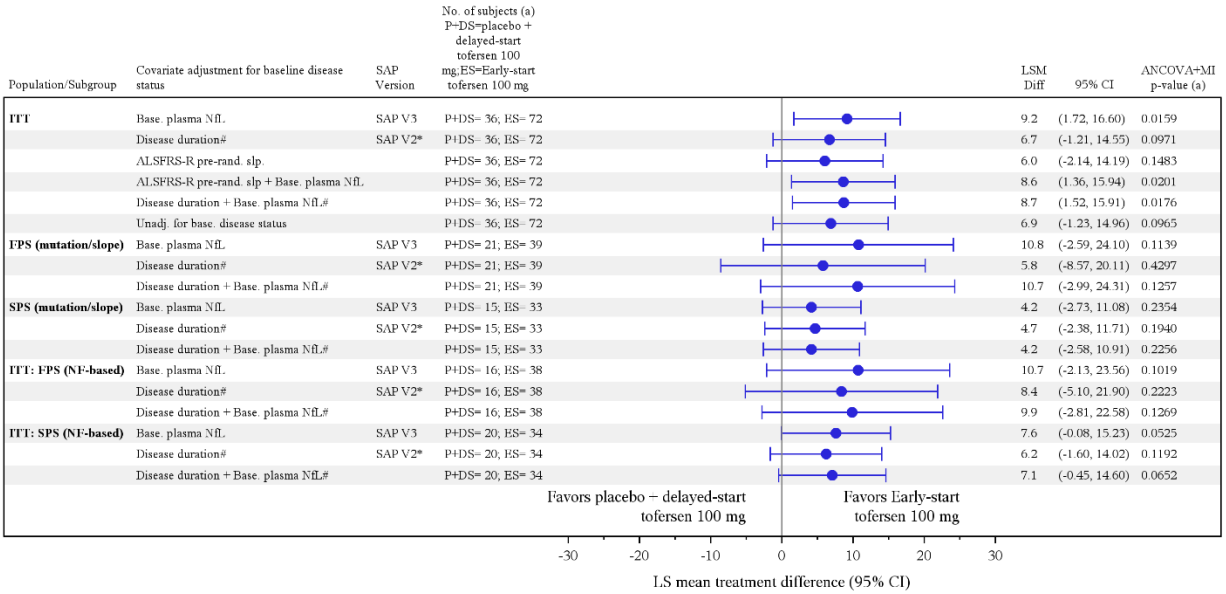
NOTE 1: Lower percent-predicted SVC depicts a worsening. Modified analyses are conducted for VALOR; analysis for VALOR and OLE is based on January 2022 data cutoff and was prespecified. All p-values are nominal. The maximum (best effort) acceptable reading is used for analysis. Readings with The American Thoracic Society (ATS) Best Criteria F (failed) are considered as missing.
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 postbaseline 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 multiple imputation. 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 18: Adjusted Mean Change (± SE) in Percent-Predicted SVC from VALOR Baseline to Week 52 (VALOR + OLE; ITT Population)**




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. Readings with ATS Best criteria F (failed) are considered as missing and imputed using MI.
NOTE 3: The maximum (best effort) acceptable reading is used for analysis. 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 percent-predicted SVC, and use of riluzole or edaravone.

## Figure 19: Forest Plot of Percent-Predicted SVC Change from VALOR Baseline to Week 52 (VALOR + OLE)




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 the OLE based on 16 July 2021 data cutoff; 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 after 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 the OLE based on 16 January 2022 data cutoff.
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 percent-predicted SVC, and use of riluzole or edaravone.
NOTE 3: For the analyses including disease duration since symptom onset in FPS (mutation/slope) and SPS (mutation/slope) 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 percent-predicted SVC, and use of riluzole or edaravone. For the other analyses in FPS (mutation/slope) and SPS (mutation/slope) 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 percent-predicted SVC and use of riluzole or edaravone.
NOTE 4: FPS and SPS (mutation/slope) are disease progression subgroups based on mutation type and prerandomization slope as defined in the protocol.
NOTE 5: FPS and SPS (NF-based) are disease progression subgroups defined by the median baseline plasma NfL (>= and < Median (75.60 pg/mL)).
#The baseline ALSFRS-R is included as an additional covariate in ANCOVA.
* No statistical testing was prespecified in SAP V2.0; all p-values are post-hoc for SAP V2.0 analyses.
(a) From the listed ANCOVA analysis based on change from baseline.
P+DS = placebo + delayed-start tofersen 100 mg; ES = Early-start tofersen 100 mg.

## 4.4. HHD Megascore

### VALOR Key Secondary Efficacy Analysis

Change from baseline to Week 28 in HHD megascore was assessed in the FPS (mutation/slope) as a key secondary endpoint in VALOR. In this subgroup, a difference of 0.02 favoring tofersen was observed (95% CI: -0.21 to 0.26); nominal p = 0.8390 (ANCOVA+MI).

The difference between groups was slightly greater (0.13 difference (95% CI: -0.10 to 0.37) when analyzed in the prespecified faster-progressing subgroup defined according to baseline plasma NfL levels in which baseline characteristics were more balanced (Figure 27).

### VALOR (Week 28) and VALOR/OLE (Week 52) Analyses in the ITT Population Adjusting for Baseline NfL

Over 28 weeks in the VALOR ITT population (n = 108), tofersen-treated participants experienced less decline in HHD megascore than those on placebo (0.10 difference; nominal p = 0.1547) [Table 11].

Over 52 weeks, the early-start group experienced less decline than the placebo/delayed-start group (0.28 difference; nominal p = 0.0186) [Table 11; Figure 20].

As described in Section 2.6.1.4.5, people living with ALS experience progressive loss of muscle strength. In contrast, the early-start group experienced an apparent stabilization in muscle strength after Week 28. The placebo/delayed-start group appeared to experience stabilization of strength after Week 40, approximately 12 weeks after initiation of tofersen and the timepoint at which maximum biological activity would be expected. From baseline to Week 52, 26.9% of the early-start participants and 8.1% of the delayed-start participants experienced an increase in HHD megascore (18.8% estimated difference in proportions; 95% CI: 1.4 to 36.2).

Improvement in muscle strength is inconsistent with the natural history of ALS. For example, in the Phase 3 dexpramipexole study (EMPOWER), only 41 of 942 participants (4.4%) experienced an improvement in HHD megascore over 12 months.

Figure 21 illustrates the consistent trends favoring early-start tofersen in the ITT population and disease progression subgroups (mutation/slope and NF-based), with adjustment for different covariates.

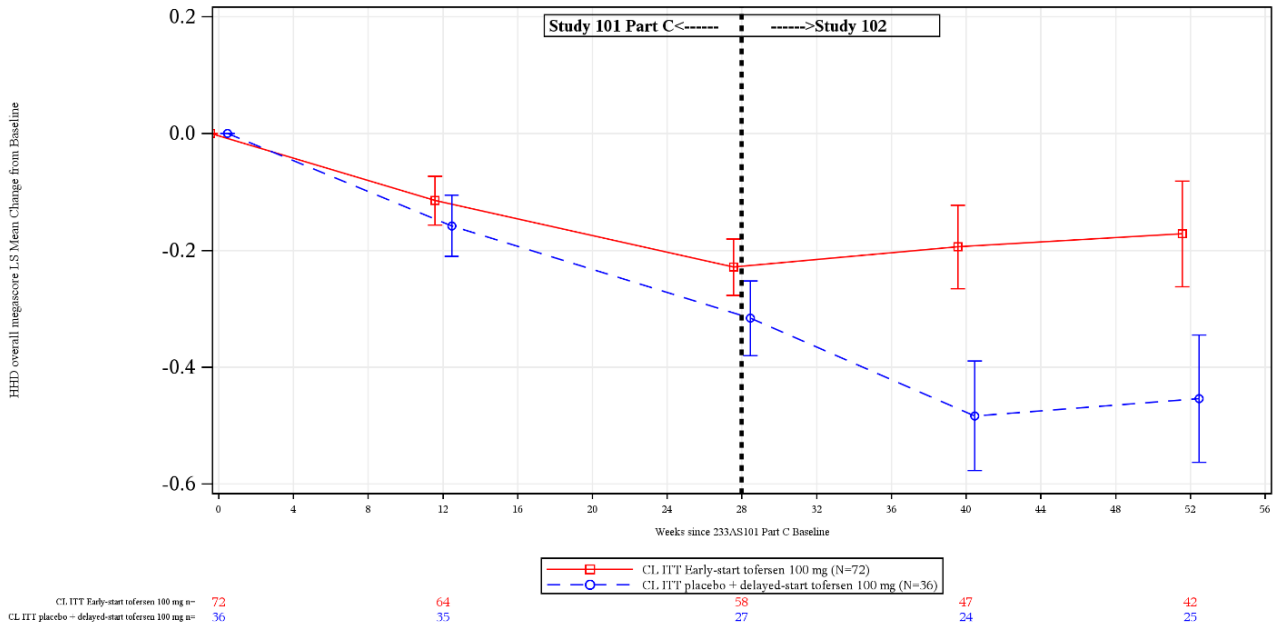
The fact that demonstrable impact on strength lags behind evidence of reduced axonal injury or neurodegeneration is consistent with the biology of ALS. For a denervated muscle to recover strength, several events must occur. First, the injured or degenerating motor neurons that are not contributing to force generation must be stabilized and re-establish neuromuscular transmission with their original myofibers. Such a neuron may then be able to contribute to reinnervation of other denervated myofibers through sprouting of collaterals and formation of new neuromuscular junctions (NMJs). As these nascent NMJs mature, the efficiency of neuromuscular transmission improves. Finally, the reinnervated myofibers need to add myofibrils to contribute additional force to the contraction of a muscle.

## Table 11: Change in HHD Megascore from VALOR Baseline (ITT Population)

| Endpoint | VALOR<br><br>Tofersen (n = 72) vs. Placebo (n = 36)<br><br>Change from Baseline to Week 28 | VALOR and OLE<br><br>Early-start Tofersen (n = 72) vs. Placebo/Delayed-start Tofersen (n = 36)<br><br>Change from Baseline to Week 52 |
|---|---|---|
| **Change from Baseline on HHD Megascore** | | |
| Adjusted means: Tof, Placebo | -0.23, -0.32 | -0.17, -0.45 |
| Tofersen-placebo: adjusted mean difference (95% CI) | 0.10 (-0.04, 0.23) | 0.28 (0.05, 0.52) |
| p-value (ANCOVA+MI) | 0.1547 | 0.0186 |

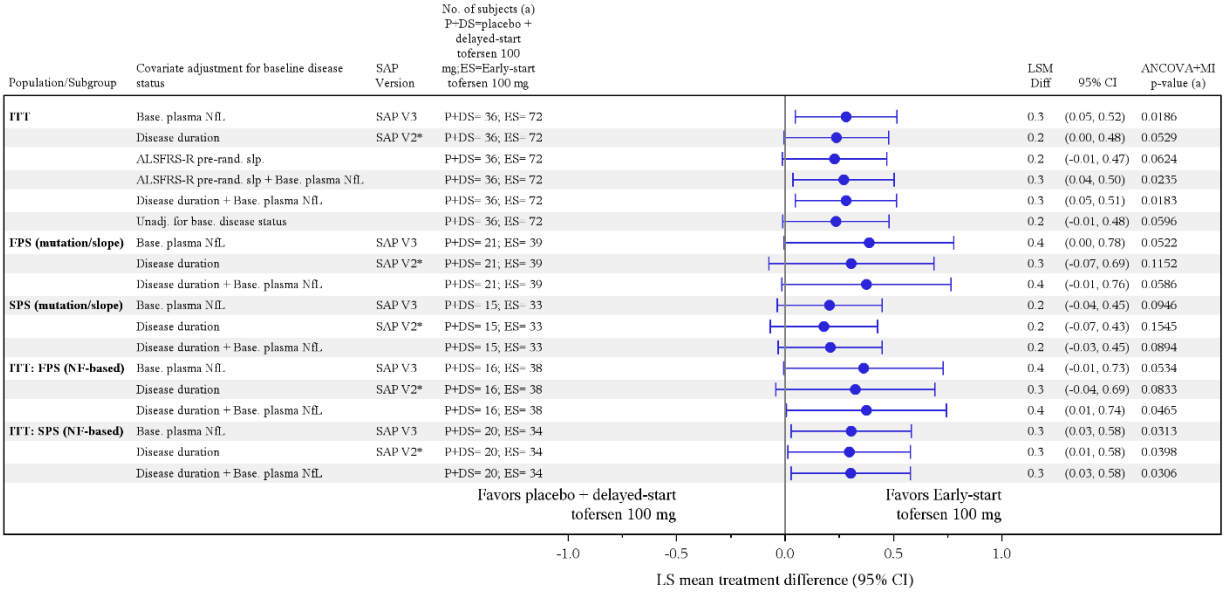
NOTE 1: A lower megascore depicts a worsening. Modified analyses are conducted for VALOR; analysis VALOR and OLE is based on the January 2022 data cutoff and was prespecified. All p-values are nominal.
NOTE 2: The multiple imputation model is based on all participants in the ITT population and includes baseline plasma NfL, treatment, use of riluzole or edaravone, relevant baseline score and postbaseline 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 multiple imputation. The ANCOVA model includes treatment as a fixed effect and adjusts for the following covariates: baseline plasma NfL, relevant baseline score, and use of riluzole or edaravone.

**Figure 20: Adjusted Mean Change (± SE) in HHD Megascore from VALOR Baseline to Week 52 (VALOR + OLE; ITT Population)**




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: The overall megascore calculated as an average normalized Z scores across the 16 muscles. 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 HHD overall megascore, and use of riluzole or edaravone.

## Figure 21: Forest Plot of HHD Megascore Change from VALOR Baseline to Week 52 (VALOR + OLE)




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 the OLE based on 16 July 2021 data cutoff; 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 the OLE based on 16 January 2022 data cutoff.
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 HHD overall megascore, and use of riluzole or edaravone.
NOTE 3: For the analyses including baseline disease duration since symptom onset in FPS (mutation/slope) and SPS (mutation/slope) 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 HHD overall megascore and use of riluzole or edaravone. For the other analyses in FPS (mutation/slope) and SPS (mutation/slope) 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 HHD overall megascore and use of riluzole or edaravone.
NOTE 4: FPS and SPS (mutation/slope) are disease progression subgroups based on mutation type and prerandomization slope as defined in the protocol.
NOTE 5: FPS and SPS (NF-based) are disease progression subgroups defined by the median baseline plasma NfL (>= and < Median (75.60 pg/mL)).
* No statistical testing was prespecified in SAP V2.0; all p-values are post-hoc for SAP V2.0 analyses.
(a) From the listed ANCOVA analysis based on change from baseline.
P+DS = placebo + delayed-start tofersen 100 mg; ES = Early-start tofersen 100 mg.

## 4.5. Effect of Tofersen on Survival

### VALOR Key Secondary Efficacy Analysis

Median time to death or PV and median time to death were assessed as key secondary endpoints in VALOR; neither were estimable in VALOR due to the limited number of events that occurred over the course of the study.

### Longer-Term Assessment of Event-Free Survival/Overall Survival in VALOR and its OLE

Following the learnings around neurofilament as a prognostic factor for disease progression, when analyzing survival data, stratified log-rank test based on baseline median NfL was conducted and baseline NfL was included as a covariate in the Cox regression model.

As of the 16 January 2022 data cutoff, all participants enrolled in VALOR had the opportunity for at least 1 year of follow-up (median: 2.3 years; range: 1 to 2.8 years). Despite this duration of follow-up, there were a limited number of death-equivalent events, thus precluding estimation of the median time to event in both treatment groups (Figure 22).

The risk of death or PV was reduced by 64%, and the risk of death was reduced by 73% in the early-start group compared to the delayed-start group (Table 12).

Analyses of time to death incorporating vital status information collected after withdrawal from the study and time to death, PV, or withdrawal due to disease progression provide consistent, supportive evidence (Table 12).

## Table 12: Time-to-Event Analyses - ITT Population

| Endpoint | VALOR and OLE (ITT population) | |
|---|---|---|
| | Early-start Tofersen 100 mg (n = 72) | Placebo/Delayed-start Tofersen 100 mg (n = 36) |
| **Time to Death or PV** | | |
| Number of events/Total number participants (%) | 12/72 (16.7%) | 8/36 (22.2%) |
| Hazard ratio (95% CI)[a] | | 0.36 (0.137, 0.941) |
| Cox regression p-value[a] | | 0.0373 |
| Log-rank test p-value[b] | | 0.0687 |
| **Time to Death** | | |
| Number of events/Total number participants (%) | 8/72 (11.1%) | 6/36 (16.7%) |
| Hazard ratio (95% CI) [a] | | 0.27 (0.084, 0.890) |
| Cox regression p-value [a] | | 0.0313 |
| Log-rank test p-value [b] | | 0.0879 |
| **Time to Death with Additional Postwithdrawal Vital status Data** [c] | | |
| Number of events/Total number participants (%) | 12/72 (16.7%) | 11/36 (30.6%) |
| Hazard ratio (95% CI) [a] | | 0.24 (0.096, 0.602) |
| Cox regression p-value [a] | | 0.0023 |
| Log-rank test p-value [b] | | 0.0096 |
| **Time to Death, PV, or Withdrawal Due to Disease Progression** | | |
| Number of events/Total number participants (%) | 18/72 (25.0%) | 13/36 (36.1%) |
| Hazard ratio (95% CI) [a] | | 0.38 (0.180, 0.821) |
| Cox regression p-value [a] | | 0.0135 |
| Log-rank test p-value [b] | | 0.0217 |

[a] Based on a Cox proportional hazards model adjusted for baseline plasma NfL, and riluzole or edaravone use

[b] Based on a log-rank test stratified by median baseline plasma NfL.

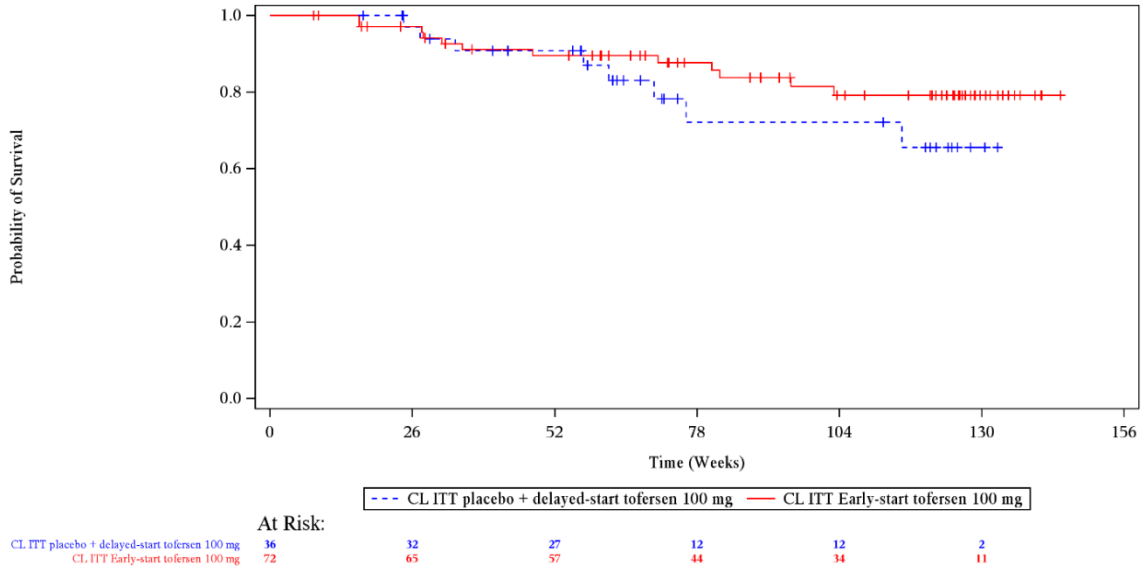
[c] Analysis incorporates vital status data obtained after discontinuation from VALOR or the OLE for participants who discontinued for reasons other than death.

NOTE 1: Analysis for VALOR and OLE is based on the January 2022 data cutoff and was prespecified. All p-values are nominal.

NOTE 2: Time to death or permanent ventilation is defined as the time from first dose to death or PV (≥ 22 hours of mechanical ventilation (invasive or noninvasive) per day for ≥21 consecutive days), whichever comes first. Participants who do not meet the endpoint definition are censored at the participant's last known alive date. Similarly, time to death, PV, or withdrawal due to disease progression is defined from first dose to first of these events. Only events that were adjudicated by the Endpoint Adjudication Committee are included for these analyses. Withdrawal due to disease progression is based on the Investigator assessment reported on the end of study case report form.

NOTE 3: Median time was not estimable.

**Figure 22: Kaplan-Meier Plot of Time to Death or Permanent Ventilation (VALOR + OLE; ITT Population)**




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

While the median time to event is not estimable in the full population, the median time to death or withdrawal due to disease progression is estimable in the subgroup of A5V carriers enrolled in VALOR. The A5V mutation is consistently associated with a rapidly progressing disease course, with a median disease duration at or less than 1.2 years across available literature [Bali 2017; Cudkowicz 1997]. As of the 16 January 2022 data cutoff, the median survival (descriptive median time from onset of symptoms to death, withdrawal, or data cutoff for those ongoing) in participants with A5V participants who received at least 1 dose of tofersen (n = 16) was 1.73 years. This is a conservative representation of the median survival due to censoring of participants who were ongoing in the study at the time of the cutoff (n = 3 [19%] with disease durations ranging from 1.89 to 3.68 years) and of those who withdrew from the study (n = 7 [44%]). As of the January cutoff, the median survival in the early-start group was 1.9 years, a 50% increase compared to the 1.3-year median observed in the delayed-start group.

## 4.6. Participant-Reported Quality of Life

Effects observed across exploratory participant-reported QoL measures, including ALSAQ-5, FSS, and EuroQoL 5 Dimension (EQ-5D), further substantiate the effects observed on the objective clinical outcome measures (Table 13).

**Table 13: Change in QoL Measures from VALOR Baseline - ITT Population**

| Endpoint | VALOR<br><br>Tofersen (n = 72) vs. Placebo (n = 36)<br><br>Change from Baseline to Week 28 | VALOR and OLE<br>(ISE SAP V3.0)<br>Early-start Tofersen (n = 72) vs. Placebo/Delayed-start tofersen (n = 36)<br>Change from Baseline to Week 52 |
|---|---|---|
| **Change from Baseline on ALSAQ-5** | | |
| Adjusted means: Tof, placebo | 6.9, 12.6 | 9.6, 19.9 |
| Tofersen-placebo: adjusted mean (95% CI) | -5.7 (-11.8, 0.4) | -10.3 (-17.33, -3.20) |
| p-value (ANCOVA/MI) | 0.0668 | 0.0044 |
| **Change from Baseline on FSS** | | |
| Adjusted means: Tof, placebo | 3.9, 6.3 | 1.3, 5.1 |
| Tofersen-placebo: adjusted mean (95% CI) | -2.4 (-7.5, 2.6) | -3.8 (-9.03, 1.38) |
| p-value (ANCOVA/MI) | 0.3365 | 0.1493 |
| **Change from Baseline on EQ-5D-5L Utility Score** | | |
| Adjusted means: Tof, placebo | -0.08, -0.21 | -0.1, -0.3 |
| Tofersen-placebo: adjusted mean (95% CI) | 0.14 (0.05, 0.23) | 0.2 (0.13, 0.32) |
| p-value (ANCOVA/MI) | 0.0029 | <0.0001 |
| **Change from Baseline on EQ-5D-VAS** | | |
| Adjusted means: Tof, placebo | -7.6, -13.6. | -7.0, -12.9 |
| Tofersen-placebo: adjusted mean (95% CI) | 6.0 (-0.7, 12.8) | 5.9 (-1.54, 13.25) |
| p-value (ANCOVA/MI) | 0.0803 | 0.1209 |

NOTE 1: Higher scores on ALSAQ-5 and FSS and lower scores on EQ-5D indicate worsening. Modified analyses are conducted for VALOR; analysis for VALOR and OLE is based on the January 2022 data cutoff and was prespecified. 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 postbaseline 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 multiple imputation. 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.

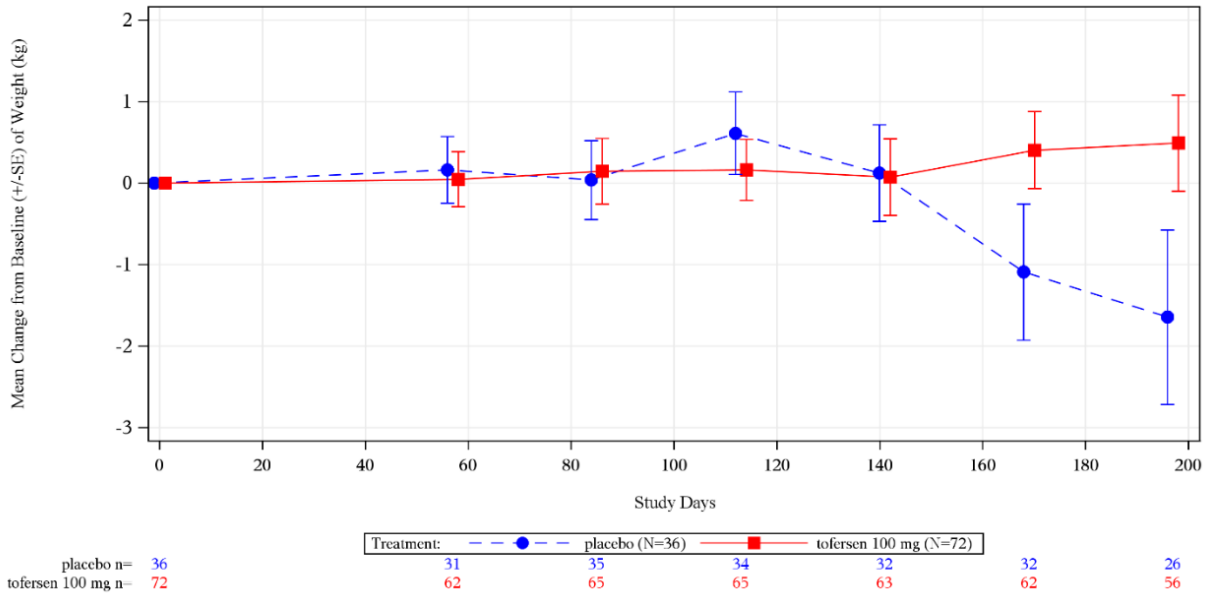
## 4.7. Weight

Weight loss has been found to be a strong independent predictor of survival in ALS. In a large population-based study comprising 2420 participants, Janse van Mantgem et al. found an adjusted HR for absolute weight loss in kilograms of 1.03 (95% CI: 1.02 to1.04, $p < 0.001$), indicating a 3% increase in the risk of death during follow-up with each additional kilogram of weight loss [Janse van Mantgem 2020].

Given this relationship, an exploratory efficacy analysis of change in weight over time was performed. Participants in the tofersen group had a lower body weight at Baseline (mean of

77.9 kg) than those in the placebo group (mean of 80.0 kg). 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]) [Figure 23].

**Figure 23: VALOR: Line Plot of Weight Mean Change from Baseline Values ± SE by Visit - ITT Population**




NOTE: 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.

## 4.8. Efficacy Conclusions

In SOD1-ALS, accumulation of toxic SOD1 protein drives loss of motor neurons which leads to progressive loss of muscle mass, strength, function, and ultimately death.

Tofersen administration lowered CSF SOD1 protein, providing indirect evidence of target engagement. This reduction of the causative protein slowed axonal injury and neurodegeneration, as indicated by reduced levels of neurofilament.

Despite clear evidence of biological activity, a statistically significant difference was not observed on the primary analysis in VALOR. This may have been related, in part, to aspects of the study design (e.g., study duration and enrichment strategy) and/or inherent challenges in a heterogeneous disease such as SOD1-ALS as detailed in Section 2.2. However, at Week 28, consistent trends favoring tofersen were observed across measures of clinical function, respiratory function, strength, and QoL. These trends were most apparent in the faster-progressing (higher baseline NfL) participants in whom there was sufficient decline in the placebo arm to observe a difference. These effects were further supported by an apparent stabilization of weight loss, which is highly clinically meaningful in ALS.

By Week 52, nominally statistically significant differences favoring earlier tofersen initiation were observed across clinical outcome measures of clinical function (ALSFRS-R), respiratory

strength (SVC), muscle strength (HHD), and patient-reported outcomes of disease severity and QoL (ALSAQ-5, EQ-5D-5L). While the limited number of events in both groups precludes estimation of median time to death or PV, available data provide early evidence of a reduced risk of death and/or PV with earlier tofersen initiation. These differences favoring early-start tofersen were discernable irrespective of which population (ITT or subgroups) was analyzed and which covariates incorporated.

The consistency of effect across measures and the temporal relationship between biological and clinical effects (e.g., CSF SOD1 lowering followed by neurofilament reductions preceding discernable clinical benefit) provide evidence that tofersen has a clinically relevant impact on disease progression in people living with SOD1-ALS. Furthermore, a statistical model built upon data from VALOR and its OLE supports that early tofersen-driven reductions in plasma NfL are directly associated with a slowing in decline in clinical function, strength, and QoL and a reduced risk of death-equivalent events over time.

Taken together, these data support that tofersen-driven reductions in neurofilament are reasonably likely to predict clinical benefit in SOD1-ALS.

# 5. SUMMARY OF MAJOR SAFETY FINDINGS

The results of the ISS analysis demonstrate that administration of tofersen 100 mg IT every 4 weeks after 3 loading doses once every 2 weeks has an acceptable safety profile in participants with SOD1-ALS.

The focus of the safety evaluation presented in this briefing book is on 2 main pools of safety data. The results from VALOR define the safety profile of tofersen 100 mg IT (n = 72) compared with placebo (n = 36). Integrated safety data from Study 101 Part B (MAD), VALOR, and the OLE (n = 147) evaluate the longer-term safety of tofersen 100 mg IT as of 15 July 2022. In context of the rarity of SOD1-ALS, the safety database is considered adequate to allow for a thorough assessment of the safety of tofersen.

## 5.1. Exposure

The safety profile of tofersen 100 mg IT was assessed in 108 participants in the completed placebo-controlled VALOR, of whom 72 participants received tofersen 100 mg and 36 participants received placebo (data cutoff 16 July 2021).

In addition, the safety profile of tofersen was assessed in integrated data for VALOR and the OLE in 147 participants who received tofersen 100 mg for 312.56 person-years as of 15 July 2022. The median exposure to tofersen 100 mg for this group was 119.4 weeks (Table 14).

**Table 14: Exposure to Tofersen 100 mg**

| | Total Duration of Exposure (Weeks) | |
|---|---|---|
| | **VALOR Tofersen 100 mg n = 72** | **Study 101 and OLE Integrated Tofersen 100 mg n = 147** |
| **Median** | 28.1 | 119.4 |
| **Min, Max** | 8, 34 | 4, 212 |

## 5.2. Summary of Adverse Events

### 5.2.1. Overview of AEs

Most participants in VALOR and the OLE experienced adverse events (AEs) [Table 15]. Across both studies, most AEs were mild to moderate in severity and did not lead to tofersen discontinuation or dose interruption. Specific AEs, including lumbar puncture-related events, SAEs, and events with fatal outcome, are discussed below.

**Table 15: Overview of AEs**

| | Participants, n (%) | | |
|---|---|---|---|
| | VALOR | | Study 101 and OLE Integrated[a] |
| | Tofersen 100 mg n = 72 | Placebo n = 36 | Tofersen 100 mg n = 147 |
| **≥1 AE** | 69 (96) | 34 (94) | 145 (99) |
| **AEs related to lumbar puncture**[b] | 58 (81) | 29 (81) | 125 (85) |
| **Grade ≥3 AEs** | 12 (17) | 4 (11) | 58 (39) |
| **Serious AEs** | 13 (18) | 5 (14) | 59 (40) |
| **AEs leading to drug discontinuation** | 4 (6) | 0 | 26 (18) |
| **AEs with fatal outcome** | 1 (1) | 0 | 19 (13) |

[a] An event in a placebo participant during VALOR is only counted once; an event in a tofersen participant during VALOR is counted in both the VALOR tofersen 100 mg column, and again in the Study 101 and OLE Integrated column

[b] Relatedness assessed by the Investigator.

### 5.2.2. Most Common AEs

Many of the commonly reported AEs in participants treated with tofersen in the clinical program were consistent with events occurring in the natural history of ALS, common conditions in the general population, or events observed in the context of lumbar puncture (Table 16).

In the placebo-controlled experience (VALOR), the most frequently reported AEs in at least 10% of participants who received tofersen 100 mg were procedural pain, headache, pain in extremity, fall, back pain, post-lumbar puncture syndrome, fatigue, arthralgia, myalgia, and nausea. The most common AEs remained similar in the open-label period.

## Table 16: AEs Reported in at Least 10% of Participants in Any Tofersen 100 mg Group

| | Participants, n (%) | | |
|---|---|---|---|
| | VALOR | | Study 101 and OLE Integrated |
| | Tofersen 100 mg n = 72 | Placebo n = 36 | Tofersen 100 mg n = 147 |
| 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) |
| CSF protein increased | 6 (8.3) | 1 (2.8) | 36 (24.5) |
| Post lumbar puncture syndrome | 13 (18.1) | 11 (30.6) | 34 (23.1) |
| 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) |
| 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) |
| CSF white blood cell count increased | 7 (9.7) | 0 | 24 (16.3) |
| Contusion | 3 (4.2) | 1 (2.8) | 24 (16.3) |
| Nasopharyngitis | 2 (2.8) | 7 (19.4) | 24 (16.3) |
| Pyrexia | 3 (4.2) | 1 (2.8) | 23 (15.6) |
| Respiratory failure | 3 (4.2) | 0 | 20 (13.6) |
| Dyspnoea | 4 (5.6) | 5 (13.9) | 19 (12.9) |
| 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) |
| Upper respiratory tract infection | 5 (6.9) | 2 (5.6) | 15 (10.2) |
| Pain | 7 (9.7) | 0 | 15 (10.2) |

NOTE 1: AEs are presented by descending frequency in the Study 101 and OLE integrated column
NOTE 2: AEs presented by PTs according to MedDRA version 25.0
NOTE 3: COVID-19 = Coronavirus Disease 2019

In VALOR, the following AEs occurred with a > 5% higher incidence in the tofersen 100 mg-treated group the in the placebo group: pain in extremity, back pain, fatigue, arthralgia, myalgia, CSF white blood cells (WBCs) count increased, pain, CSF protein increased, musculoskeletal stiffness and neuralgia. In addition, the preferred term (PT) of pleocytosis (a term that reflects the same clinical concept as CSF WBCs count increased) was reported in 3 participants (4.2%).

### 5.2.3. AEs Related to Lumbar Puncture

AEs associated with the administration of tofersen by lumbar puncture, such as procedural pain, headache, back pain, and post-lumbar puncture syndrome, have been observed.

Most lumbar puncture-related events were classified as mild or moderate in severity and were not treatment limiting. The incidence and severity of these events were consistent with events expected to occur with lumbar puncture. No serious complications of lumbar puncture, such as serious infections, have been observed in the clinical studies

### 5.2.4. SAEs

Most SAEs observed in the tofersen clinical trials were events commonly observed in the study population, comorbidities associated with ALS, or events related to ALS progression (Table 17). The most common SAEs in the tofersen clinical trials included respiratory failure, aspiration pneumonia, dysphagia, and pulmonary embolism, all of which are commonly seen in people living with ALS.

SAEs with fatal outcome are discussed in Section 5.2.5. Neurologic SAEs, including myelitis, radiculitis, papilledema, and aseptic meningitis, which encompass most of the SAEs assessed as related to study treatment by the Investigators, are discussed in Section 5.3.

**Table 17: Summary of SAEs Reported in at Least 2 Participants in the Integrated Dataset**

| | Participants, n (%) | | |
|---|---|---|---|
| | VALOR | | Study 101 and OLE Integrated |
| | Tofersen 100 mg n = 72 | Placebo n = 36 | Tofersen 100 mg n = 147 |
| **Any serious AE** | **13 (18.1)** | **5 (13.9)** | **59 (40.1)** |
| Respiratory failure | 1 (1.4) | 0 | 16 (10.9) |
| Pneumonia aspiration | 2 (2.8) | 0 | 10 (6.8) |
| Dysphagia | 0 | 0 | 7 (4.8) |
| Pulmonary embolism | 3 (4.2) | 1 (2.8) | 6 (4.1) |
| Acute respiratory failure | 1 (1.4) | 0 | 5 (3.4) |
| Pneumonitis aspiration | 2 (2.8) | 0 | 4 (2.7) |
| Pneumonia | 0 | 0 | 3 (2.0) |
| Fall | 0 | 0 | 3 (2.0) |
| Intracranial pressure increased | 0 | 0 | 3 (2.0) |
| Respiratory arrest | 0 | 0 | 2 (1.4) |
| Respiratory distress | 0 | 0 | 2 (1.4) |
| Chronic respiratory failure | 0 | 0 | 2 (1.4) |
| COVID-19 | 0 | 0 | 2 (1.4) |
| Myelitis | 1 (1.4) | 0 | 2 (1.4) |
| Septic shock | 0 | 0 | 2 (1.4) |
| Faecaloma | 1 (1.4) | 0 | 2 (1.4) |
| Amyotrophic lateral sclerosis | 0 | 0 | 2 (1.4) |
| Nephrolithiasis | 0 | 0 | 2 (1.4) |
| Back pain | 0 | 0 | 2 (1.4) |

NOTE 1: AEs are presented by descending frequency in the Study 101 and OLE integrated column
NOTE 2: AEs presented by PTs according to MedDRA version 25.0
NOTE 3: COVID-19 = Coronavirus Disease 2019

### 5.2.5. Deaths

As of 15 July 2022, 22 deaths have been reported in the tofersen clinical development program (i.e., all participants who received any dose of tofersen); 19 were in participants receiving tofersen 100 mg (i.e., all data starting at the point of 100 mg tofersen initiation). Consistent with causes of death typically observed in ALS, most of the deaths were respiratory in nature. Most deaths were associated with the underlying disease, and none were assessed as related to study treatment by the Investigator.

## 5.3. Serious Neurologic Events

Immunostimulatory and proinflammatory effects have been associated with ASOs in multiple nonclinical species. In rodents, activation of toll-like receptor 9 by ASOs can result in cytokine release, lymphoid hyperplasia, and lymphohistiocytic cell infiltrates in various organs [Frazier 2015]. In monkeys, ASO-mediated inflammation results from complement activation and similarly can cause lymphoid hyperplasia and lymphohistiocytic infiltrates. However, tofersen did not result in significant chemokine induction (interleukin-8 and interferon gamma induced protein-10) in a human cell line.

In the clinic, proinflammatory effects, such as flu-like symptoms and injection site reactions, have been seen with systemic (intravenous or subcutaneous) administration of ASOs [Bennett 2019]. It remains uncertain whether more serious inflammatory conditions, such as the vasculitis seen with some ASOs in monkeys, translate to humans [Frazier 2015].

Neurologic SAEs consistent with inflammation in the CNS have occurred in participants receiving tofersen in clinical trials (Table 18). These events have been accompanied by CSF pleocytosis and/or an increase in CSF protein. Although a large proportion of participants have had CSF laboratory abnormalities suggestive of an inflammatory process, only a small number of participants have had clinically significant events, of which still fewer required study treatment discontinuation. The mechanism of these inflammatory events and the relationship between the laboratory abnormalities and these events are not well understood.

**Table 18: Summary of Neurologic SAEs of Interest**

| | Participants, n (%) | | |
|---|---|---|---|
| | VALOR | | Study 101 and OLE Integrated |
| | Tofersen 100 mg n = 72 | Placebo n = 36 | Tofersen 100 mg n = 147 |
| **Number of Participants with Serious Neurologic Events** | **4 (5.6)** | **0** | **10 (6.8)** |
| Myelitis | 1 (1.4) | 0 | 2 (1.4) |
| Radiculopathy | 0 | 0 | 1 (0.7) |
| Lumbar radiculopathy | 1 (1.4) | 0 | 1 (0.7) |
| Myelitis transverse | 1 (1.4) | 0 | 1 (0.7) |
| Neurosarcoidosis[a] | 0 | 0 | 1 (0.7) |
| Meningitis aseptic | 0 | 0 | 1 (0.7) |
| Meningitis chemical | 1 (1.4) | 0 | 1 (0.7) |
| Intracranial pressure increased | 0 | 0 | 3 (2.0) |
| Papilloedema | 0 | 0 | 1 (0.7) |

[a] Verbatim term: Neurosarcoid transverse myelitis
NOTE: AEs presented by PTs according to MedDRA version 25.0

### 5.3.1. Myelitis and Radiculitis

Transverse myelitis is inflammation of the spinal cord, characterized by motor and sensory symptoms, and is a rare event in the general population [Brinar 2006].

A total of 6 participants across VALOR and its OLE, all receiving tofersen 100 mg, experienced SAEs of myelitis (4 participants) or radiculitis (2 participants). The Investigator considered the events related to study treatment in 5 of 6 cases. In 1 event of neurosarcoidosis (verbatim term: neurosarcoid transverse myelitis), a confounding diagnosis of sarcoidosis was present, and the event was assessed by the Investigator as unrelated to study treatment. This event led to

discontinuation of tofersen. In 1 event of myelitis, immunomodulatory treatment was initiated, tofersen was permanently discontinued and the participant withdrew from the study. The event completely resolved approximately 3 months after the last dose of tofersen, and magnetic resonance imaging (MRI) performed 6 months later demonstrated complete disappearance of the multifocal enhancement and only subtle residual abnormality in the cervical spine. Two events of myelitis (PTs myelitis transverse and myelitis) were identified based on imaging and/or laboratory findings only, without corresponding clinical signs or symptoms. Both participants have remained in the OLE as of the 15 July 2022 data cutoff.

One participant with an SAE of radiculopathy had symptoms after 24 doses of tofersen with MRI findings demonstrating cauda equina enhancement. The event resolved with sequelae after approximately 9 months. A second participant with an SAE of lumbar radiculopathy had symptoms after IT injection of tofersen, but MRI of the lumbosacral spine showed no corresponding abnormality and symptoms resolved within days. This participant had 3 subsequent nonserious events of lumbar radiculopathy, which were mild to moderate in severity and also resolved within days. Both participants with events of radiculitis/radiculopathy have remained in the OLE as of the 15 July 2022 data cutoff.

### 5.3.2. Papilledema

Papilledema is defined as optic disc swelling due to high intracranial pressure. Possible conditions causing papilledema include intracerebral mass lesions, cerebral hemorrhage, head trauma, meningitis, hydrocephalus, and spinal cord lesions. Irrespective of the cause, visual loss consequent to papilledema is a main concern with this condition [Rigi 2015].

A total of 4 participants experienced SAEs involving elevated intracranial pressure and/or papilledema (3 events with the PT of intracranial pressure increased and 1 event with the PT of papilloedema) in the OLE, all of which were considered related to study treatment by the Investigator. Despite the different terms reported in each case, all 4 participants did have papilledema.

Further review of clinical trial data identified 3 participants with nonserious AEs of papilledema. Two of these individuals also experienced SAEs of intracranial pressure increased, and 1 had a nonserious AE of intracranial pressure increased. Two additional participants had non-serious AEs of intracranial pressure increased, and 1 had a nonserious AE of CSF pressure increased (all without reported AEs of papilledema).

None of these AEs or SAEs led to discontinuation of tofersen as of the 15 July 2022 data cutoff. No similar events were seen in the placebo group in VALOR.

### 5.3.3. Aseptic Meningitis

Aseptic meningitis is defined as meningeal inflammation (i.e., CSF pleocytosis $\geq 5$ cells/mm$^3$) that is not related to an infectious process [Tattevin 2019]. In the general population, the incidence rates of aseptic meningitis range from 2.9 to 7.6 cases per 100,000 persons [Mount and Boyle 2017]. No reporting incidence rate for meningitis was identified in ALS.

Aseptic meningitis has been reported with another IT-administered ASO, nusinersen. Chemical meningitis and CSF pleocytosis have been observed in other IT-administered drugs in adults,

such as IT cytarabine [Chamberlain 2012], IT methotrexate [Jacob 2015], and tominersen [Tabrizi 2019].

In participants receiving tofersen 100 mg in VALOR, AEs of meningitis chemical (n = 1), pleocytosis (n = 3), and CSF WBCs count increased (n = 7) were observed, in contrast to no events observed in participants receiving placebo. Most events were nonserious (n = 10); however, 1 event of meningitis chemical was serious and led to discontinuation of tofersen. Of all participants who received tofersen 100 mg in Study 101 and the OLE, 13 participants experienced AEs of pleocytosis, 24 participants had AEs of CSF WBCs count increased, and 5 participants had AEs of CSF WBCs count positive. None of these events led to discontinuation of tofersen. One additional SAE of meningitis aseptic occurred in a participant in the OLE and did not lead to discontinuation of tofersen; this participant also experienced an SAE of papilledema.

The CSF profile in aseptic meningitis is frequently associated with pleocytosis [Jolles 2000], and shifts from low or normal to high in CSF WBCs count and CSF protein were observed in the majority of participants receiving tofersen (Table 19). Many of these abnormalities were not reported as AEs by the Investigators.

**Table 19: Postbaseline CSF Laboratory Abnormalities**

| | Participants, n (%) | | |
|---|---|---|---|
| | VALOR | | Study 101 and OLE Integrated |
| | Tofersen 100 mg n = 72 | Placebo n = 36 | Tofersen 100 mg n = 147 |
| **≥1 CSF WBC Value >$10\times10^{6}$/L** | 42/72 (58) | 2/36 (6) | 117/147 (80) |
| **≥1 CSF WBC Value >$5\times10^{6}$/L** | 55/72 (76) | 6/36 (17) | 134/147 (91) |
| **Proportion With Shift To High CSF Protein[a]** | 31/46 (67) | 6/20 (30) | 105/117 (90) |

[a] Shift to high includes normal to high, low to high, and unknown to high.

## 5.4. Safety Conclusions

The safety of tofersen has been well characterized in a randomized, placebo-controlled study and in an open-label study in a total of 147 participants with SOD1-ALS who received at least 1 dose of tofersen 100 mg. The overall tofersen exposure across the different clinical trials allows for an adequate assessment of safety in the context of this orphan disease, with 352.55 person-years of exposure. Data on long-term exposure are emerging. As of the 15 July 2022 data cutoff, 57 participants have had at least 144 weeks of exposure to tofersen 100 mg.

Most AEs reported during treatment with tofersen were consistent with the types and severities of events seen in SOD1-ALS, observed in the context of the lumbar puncture procedure, or occurring commonly in the general population. The most commonly observed events were mild to moderate in severity and did not lead to study treatment discontinuation or dose interruption.

AEs of pain in extremity, back pain, fatigue, arthralgia, myalgia, CSF WBCs count increased, pain, CSF protein increased, musculoskeletal stiffness and neuralgia occurred with a > 5% higher incidence in the tofersen 100 mg–treated group than in the placebo group. Although lumbar puncture-related events were common in both placebo and tofersen-treated participants, they were generally mild to moderate in severity, managed through standard of care, and not treatment limiting.

A total of 22 deaths were reported in participants treated with tofersen at any dose. Most deaths reported in participants were respiratory in nature, consistent with the causes of death commonly seen in participants with ALS. None were considered related to tofersen.

Serious neurologic events of myelitis/radiculitis, papilledema, and aseptic meningitis have been observed in 6.8% of participants who received tofersen 100 mg in the clinical studies. These events are manageable with standard of care. CSF laboratory abnormalities, including pleocytosis and elevated protein, were commonly observed. In most cases, these were asymptomatic and not treatment limiting. The relationship of these laboratory findings to serious neurological events is not well understood.

In conclusion, tofersen and its administration via lumbar puncture have been shown to be generally well tolerated with an acceptable safety profile for the treatment of SOD1-ALS.

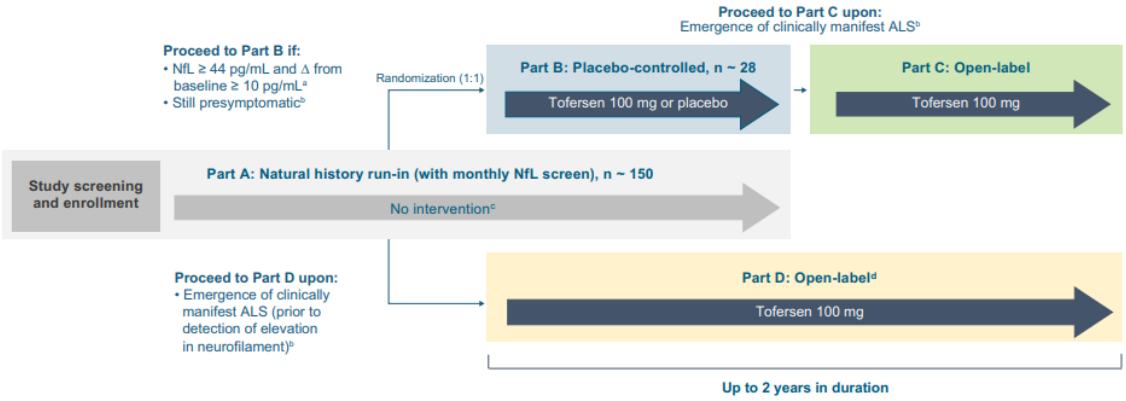
# 6. CONFIRMATORY DATA PACKAGE

Biogen is committed to confirming the clinical benefit of tofersen via the ongoing ATLAS study (Study 233AS303; NCT04856982) and supportive data from the OLE and real-world mechanisms (EAP, disease registries, natural history data sets, etc.). ATLAS is an ongoing, adequate, and well-controlled trial that is designed to evaluate the effects of tofersen when initiated in *SOD1* mutation carriers with biomarker evidence of disease activity (elevated plasma NfL) but without clinically manifest disease (Figure 24) [Benatar 2022]. The study is designed to follow 150 at-risk *SOD1* mutation carriers in a natural history run-in (Part A) where plasma NfL levels are monitored monthly. Upon detection of an above-threshold elevation in plasma NfL, participants will be screened for the placebo-controlled (1:1 randomization) study period (Part B; n = 28). Participants who develop signs or symptoms of ALS (clinically manifest ALS) will be offered the opportunity to receive open-label tofersen in Part C of the study. The primary endpoint will assess the proportion of participants who develop clinically manifest ALS at 12 months. In addition to other measures of phenoconversion (proportion who develop clinically manifest ALS at 24 months, time to emergence of clinically manifest ALS), key secondary endpoints will include changes in plasma NfL, SOD1 protein, ALSFRS-R, percent-predicted SVC, and measures of event-free survival.

Initiated in mid-2021, over 50% of the target population (n = 150) has been enrolled at sites across 14 countries to date. Based on the current study design and enrollment rate, data are expected to be available in 2027.

Additional long-term data generation plans to supplement the ATLAS study include the combined analysis of final data from VALOR and its OLE to evaluate the effects of early-start versus delayed-start tofersen on survival and function and descriptive analyses of disease duration by *SOD1* variant type in tofersen-treated versus untreated individuals based on real-world evidence (EAP, disease registries, natural history data sets, etc.).

**Figure 24: ATLAS Study Design**




[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 after Biogen's review of the results of the Phase 3 VALOR study.
Source: from [Benatar 2022]

# 7. OVERALL CONCLUSION

SOD1-ALS is a progressive disease in which motor neuron loss leads to weakness, loss of function, and ultimately death. For the estimated 330 people in the US currently living with SOD1-ALS, there remains tremendous unmet medical need [Brown 2021; Zou 2017].

In people living with SOD1-ALS, production of toxic SOD1 protein leads to axonal injury/ neuronal degeneration and death of motor neurons. When these axons are injured or degenerating, neurofilament leaks into the interstitial fluid before passing into the CSF and blood where levels can be quantified. While reductions in neurofilament may not be observed with all ALS therapies (e.g., due to different mechanisms of action), a lowering of neurofilament is generally thought to represent a slowing of axonal injury and neurodegeneration, thus providing objective evidence of treatment effect.

The main objective of the VALOR study at the outset was to demonstrate the clinical benefit of tofersen as measured by the ALSFRS-R at Week 28. We acknowledge that this objective was not achieved, as evidenced by the lack of statistical significance on the primary endpoint. Also, the reliance on open-label data to understand the potential clinical benefit, while important, has several limitations. Furthermore, although reductions in plasma NfL were prespecified as a secondary endpoint, it was done without the foreknowledge that it would form the primary basis of an NDA for accelerated approval. Despite these limitations, clinical effects of this magnitude, including some evidence of improvement, have not been observed in ALS trials to date and are completely inconsistent with the natural history of the disease.

The well-understood pathophysiology of SOD1-ALS and the mechanism of action of tofersen strengthen the biological plausibility of the position that neurofilament lowering represents reduction of the neurodegenerative process. The temporal relationship between biological effects and clinical effects further demonstrates that tofersen is having a clinically relevant impact on disease progression in SOD1-ALS. Modeling based on data from VALOR and its OLE demonstrates the relationship between reductions in neurofilament and clinical effects. Taken together, these observations support that tofersen-driven reductions in neurofilament are reasonably likely to predict clinical benefit in SOD1-ALS.

Biogen is committed to confirming the clinical benefit of tofersen via the ongoing ATLAS study and supportive data from the OLE and real-world mechanisms (EAP, disease registries, natural history data sets, etc).

# 8. REFERENCES

Abu-Rumeileh S, Vacchiano V, Zenesini C, et al. Diagnostic-prognostic value and electrophysiological correlates of CSF biomarkers of neurodegeneration and neuroinflammation in amyotrophic lateral sclerosis. J Neurol. 2020;267(6):1699-1708. Epub 20200225.

ALSoD. Amyotrophic Lateral Sclerosis Online Database - ALSoD. Institute of Psychiatry Psychology and Neuroscience (IoPPN). Published 2023 [Accessed January 2023].

Andrews JA, Meng L, Kulke SF, et al. Association Between Decline in Slow Vital Capacity and Respiratory Insufficiency, Use of Assisted Ventilation, Tracheostomy, or Death in Patients With Amyotrophic Lateral Sclerosis. JAMA Neurol. 2018;75(1):58-64.

Bakers JNE, de Jongh AD, Bunte TM, et al. Using the ALSFRS-R in multicentre clinical trials for amyotrophic lateral sclerosis: potential limitations in current standard operating procedures. Amyotroph Lateral Scler Frontotemporal Degener. 2021:1-8. Epub 20211224.

Bali T, Self W, Liu J, et al. Defining SOD1 ALS natural history to guide therapeutic clinical trial design. J Neurol Neurosurg Psychiatry. 2017;88(2):99-105. Epub 2016/06/03.

Baumann F, Henderson RD, Morrison SC, et al. Use of respiratory function tests to predict survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2010;11(1-2):194-202.

Bedlack RS, Vaughan T, Wicks P, et al. How common are ALS plateaus and reversals? Neurology. 2016;86(9):808-12. Epub 20151209.

Benatar M, Turner MR, Wuu J. Defining pre-symptomatic amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener. 2019;20(5-6):303-309. Epub 2019/03/20.

Benatar M, Wuu J, Andersen PM, et al. Randomized, double-blind, placebo-controlled trial of arimoclomol in rapidly progressive SOD1 ALS. Neurology. 2018a;90(7):e565-e574. Epub 2018/01/24.

Benatar M, Wuu J, Andersen PM, et al. Design of a Randomized, Placebo-Controlled, Phase 3 Trial of Tofersen Initiated in Clinically Presymptomatic SOD1 Variant Carriers: the ATLAS Study. Neurotherapeutics. 2022;19(4):1248-1258. Epub 20220518.

Benatar M, Wuu J, Andersen PM, et al. Neurofilament light: A candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. Ann Neurol. 2018b;84(1):130-139. Epub 2018/08/16.

Benatar M, Zhang L, Wang L, et al. Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS. Neurology. 2020;95(1):e59-e69. Epub 20200508.

Bennett CF. Therapeutic Antisense Oligonucleotides Are Coming of Age. Annu Rev Med. 2019;70:307-321.

Brettschneider J, Petzold A, Süssmuth SD, et al. Axonal damage markers in cerebrospinal fluid are increased in ALS. Neurology. 2006;66(6):852-6.

Bridel C, van Wieringen WN, Zetterberg H, et al. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. JAMA Neurol. 2019;76(9):1035-1048.

Brinar VV, Habek M, Brinar M, et al. The differential diagnosis of acute transverse myelitis. Clin Neurol Neurosurg. 2006;108(3):278-83. Epub 20051220.

Brodovitch A, Boucraut J, Delmont E, et al. Combination of serum and CSF neurofilament-light and neuroinflammatory biomarkers to evaluate ALS. Sci Rep. 2021;11(1):703. Epub 20210112.

Brown CA, Lally C, Kupelian V, et al. Estimated Prevalence and Incidence of Amyotrophic Lateral Sclerosis and SOD1 and C9orf72 Genetic Variants. Neuroepidemiology. 2021:1-12. Epub 20210709.

Brown RH, Al-Chalabi A. Amyotrophic Lateral Sclerosis. N Engl J Med. 2017;377(2):162-172.

Bruneteau G, Bauché S, Gonzalez de Aguilar JL, et al. Endplate denervation correlates with Nogo-A muscle expression in amyotrophic lateral sclerosis patients. Ann Clin Transl Neurol. 2015;2(4):362-72. Epub 20150216.

Bunton-Stasyshyn RK, Saccon RA, Fratta P, et al. SOD1 Function and Its Implications for Amyotrophic Lateral Sclerosis Pathology: New and Renascent Themes. Neuroscientist. 2015;21(5):519-29. Epub 2014/12/09.

Cedarbaum JM, Stambler N, Malta E, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). Journal of the neurological sciences. 1999;169(1-2):13-21.

Chamberlain MC. Neurotoxicity of Intra-CSF Liposomal Cytarabine (DepoCyt) Administered for the Treatment of Leptomeningeal Metastases: A Retrospective Case Series. J Neurooncol. 2012;109(1):143-8. Epub 2012/04/27.

Chiò A, Logroscino G, Hardiman O, et al. Prognostic factors in ALS: A critical review. Amyotroph Lateral Scler. 2009;10(5-6):310-23.

Coppedè F, Stoccoro A, Mosca L, et al. Increase in DNA methylation in patients with amyotrophic lateral sclerosis carriers of not fully penetrant SOD1 mutations. Amyotroph Lateral Scler Frontotemporal Degener. 2018;19(1-2):93-101. Epub 2017/09/01.

Cudkowicz ME, McKenna-Yasek D, Sapp PE, et al. Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Ann Neurol. 1997;41(2):210-21.

Cudkowicz ME, van den Berg LH, Shefner JM, et al. Dexpramipexole versus placebo for patients with amyotrophic lateral sclerosis (EMPOWER): a randomised, double-blind, phase 3 trial. Lancet Neurol. 2013;12(11):1059-67. Epub 2013/09/23.

Darras BT, Farrar MA, Mercuri E, et al. An Integrated Safety Analysis of Infants and Children with Symptomatic Spinal Muscular Atrophy (SMA) Treated with Nusinersen in Seven Clinical Trials. CNS Drugs. 2019;33(9):919-932.

De Schaepdryver M, Goossens J, De Meyer S, et al. Serum neurofilament heavy chains as early marker of motor neuron degeneration. Ann Clin Transl Neurol. 2019a;6(10):1971-1979. Epub 2019/09/13.

De Schaepdryver M, Goossens J, Jeromin A, et al. Analytical performance of a CE-marked immunoassay to quantify phosphorylated neurofilament heavy chains. Clin Chem Lab Med. 2019b;57(8):e199-e202.

De Schaepdryver M, Lunetta C, Tarlarini C, et al. Neurofilament light chain and C reactive protein explored as predictors of survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2020;91(4):436-437. Epub 20200206.

Delaby C, Alcolea D, Carmona-Iragui M, et al. Differential levels of Neurofilament Light protein in cerebrospinal fluid in patients with a wide range of neurodegenerative disorders. Sci Rep. 2020;10(1):9161. Epub 20200608.

European Medicines Agency. Guideline on clinical investigation of medicinal products for the treatment of amyotrophic lateral sclerosis (ALS).

Falzone YM, Domi T, Agosta F, et al. Serum phosphorylated neurofilament heavy-chain levels reflect phenotypic heterogeneity and are an independent predictor of survival in motor neuron disease. J Neurol. 2020;267(8):2272-2280. Epub 20200418.

Falzone YM, Domi T, Mandelli A, et al. Integrated evaluation of a panel of neurochemical biomarkers to optimize diagnosis and prognosis in amyotrophic lateral sclerosis. Eur J Neurol. 2022;29(7):1930-1939. Epub 20220323.

Food and Drug Administration. Guidance for Industry - Expedited Programs for Serious Conditions - Drugs and Biologics.

Food and Drug Administration. Amyotrophic Lateral Sclerosis: Developing Drugs for Treatment  Guidance for Industry.

Feneberg E, Oeckl P, Steinacker P, et al. Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. Neurology. 2018;90(1):e22-e30. Epub 20171206.

Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med. 2017;377(18):1723-1732.

Fischer LR, Culver DG, Tennant P, et al. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. Exp Neurol. 2004;185(2):232-40.

Fournier C. ROADS to a better ALS outcome measure - the new Rasch-Built Overall ALS Disability Scale (ROADS). Emory University; 2020. p. 1-33.

Franchignoni F, Mora G, Giordano A, et al. Evidence of multidimensionality in the ALSFRS-R Scale: a critical appraisal on its measurement properties using Rasch analysis. J Neurol Neurosurg Psychiatry. 2013;84(12):1340-5. Epub 20130320.

Frazier KS. Antisense oligonucleotide therapies: the promise and the challenges from a toxicologic pathologist's perspective. Toxicol Pathol. 2015;43(1):78-89. Epub 2014/11/09.

Gaetani L, Blennow K, Calabresi P, et al. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019;90(8):870-881. Epub 2019/04/09.

Gaiani A, Martinelli I, Bello L, et al. Diagnostic and Prognostic Biomarkers in Amyotrophic Lateral Sclerosis: Neurofilament Light Chain Levels in Definite Subtypes of Disease. JAMA Neurol. 2017;74(5):525-532.

Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS One. 2013;8(9):e75091. Epub 2013/09/20.

Gille B, De Schaepdryver M, Goossens J, et al. Serum neurofilament light chain levels as a marker of upper motor neuron degeneration in patients with Amyotrophic Lateral Sclerosis. Neuropathol Appl Neurobiol. 2019;45(3):291-304. Epub 20180718.

Gołąb-Janowska M, Honczarenko K, Stankiewicz J. Usefulness of the ALSAQ-5 scale in evaluation of quality of life in amyotrophic lateral sclerosis. Neurol Neurochir Pol. 2010;44(6):560-6.

Gordon PH, Cheng B, Salachas F, et al. Progression in ALS is not linear but is curvilinear. J Neurol. 2010;257(10):1713-7.

Gordon PH, Salachas F, Lacomblez L, et al. Predicting survival of patients with amyotrophic lateral sclerosis at presentation: a 15-year experience. Neurodegener Dis. 2013;12(2):81-90. Epub 2012/08/21.

Halstead LS. Post-polio syndrome. Sci Am. 1998;278(4):42-7.

Jacob LA, Sreevatsa A, Chinnagiriyappa LK, et al. Methotrexate-induced chemical meningitis in patients with acute lymphoblastic leukemia/lymphoma. Ann Indian Acad Neurol. 2015;18(2):206-9.

Janse van Mantgem MR, van Eijk RPA, van der Burgh HK, et al. Prognostic value of weight loss in patients with amyotrophic lateral sclerosis: a population-based study. J Neurol Neurosurg Psychiatry. 2020;91(8):867-875. Epub 20200623.

Jolles S, Sewell WA, Leighton C. Drug-induced aseptic meningitis: diagnosis and management. Drug Saf. 2000;22(3):215-26.

Kaufmann P, Levy G, Thompson JL, et al. The ALSFRSr predicts survival time in an ALS clinic population. Neurology. 2005;64(1):38-43.

Khalil M, Pirpamer L, Hofer E, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat Commun. 2020;11(1):812. Epub 20200210.

Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018;14(10):577-589.

Kiebert GM, Green C, Murphy C, et al. Patients' Health-related Quality of Life and Utilities Associated with Different Stages of Amyotrophic Lateral Sclerosis. Journal of the neurological sciences. 2001;191(1-2):87-93.

Krupp LB, LaRocca NG, Muir-Nash J, et al. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Archives of Neurology. 1989;46(10):1121-1123.

Lechtzin N, Cudkowicz ME, de Carvalho M, et al. Respiratory measures in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener. 2018;19(5-6):321-330. Epub 2018/03/23.

Lou JS, Moore D, Gordon PH, et al. Correlates of quality of life in ALS: Lessons from the minocycline study. Amyotroph Lateral Scler. 2010;11(1-2):116-21.

Lu CH, Macdonald-Wallis C, Gray E, et al. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. Neurology. 2015;84(22):2247-57. Epub 20150501.

Manouchehrinia A, Piehl F, Hillert J, et al. Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. Ann Clin Transl Neurol. 2020;7(1):139-143. Epub 20200101.

McCampbell A, Cole T, Wegener AJ, et al. Antisense oligonucleotides extend survival and reverse decrement in muscle response in ALS models. J Clin Invest. 2018;128(8):3558-3567. Epub 2018/07/16.

McComas AJ, Sica RE, Currie S. An electrophysiological study of Duchenne dystrophy. J Neurol Neurosurg Psychiatry. 1971;34(4):461-8.

McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244(22):6049-55.

McElhiney MC, Rabkin JG, Gordon PH, et al. Prevalence of fatigue and depression in ALS patients and change over time. J Neurol Neurosurg Psychiatry. 2009;80(10):1146-9.

Millecamps S, Salachas F, Cazeneuve C, et al. SOD1, ANG, VAPB, TARDBP, and FUS mutations in familial amyotrophic lateral sclerosis: genotype-phenotype correlations. J Med Genet. 2010;47(8):554-60. Epub 20100624.

Miller RG, Mitchell JD, Lyon M, et al. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database Syst Rev. 2002(2):CD001447.

Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS. The New England Journal of Medicine. 2020;383(2):109-119.

Mitsumoto H, Brooks BR, Silani V. Clinical trials in amyotrophic lateral sclerosis: why so many negative trials and how can trials be improved? Lancet Neurol. 2014;13(11):1127-1138.

Mount HR, Boyle SD. Aseptic and Bacterial Meningitis: Evaluation, Treatment, and Prevention. Am Fam Physician. 2017;96(5):314-322.

Nicholson K, Murphy A, McDonnell E, et al. Improving symptom management for people with amyotrophic lateral sclerosis. Muscle Nerve. 2018;57(1):20-24. Epub 20170701.

Ohashi Y, Tashiro K, Itoyama Y, et al. [Study of functional rating scale for amyotrophic lateral sclerosis: revised ALSFRS(ALSFRS-R) Japanese version]. No To Shinkei. 2001;53(4):346-55.

Olsson B, Alberg L, Cullen NC, et al. NFL is a marker of treatment response in children with SMA treated with nusinersen. J Neurol. 2019 Epub 2019/05/23.

Paganoni S, Cudkowicz M, Berry JD. Outcome measures in amyotrophic lateral sclerosis clinical trials. Clin Investig (Lond). 2014;4(7):605-618.

Paganoni S, Macklin EA, Hendrix S, et al. Trial of Sodium Phenylbutyrate-Taurursodiol for Amyotrophic Lateral Sclerosis. N Engl J Med. 2020;383(10):919-930.

Pinto S, de Carvalho M. Comparison of slow and forced vital capacities on ability to predict survival in ALS. Amyotroph Lateral Scler Frontotemporal Degener. 2017;18(7-8):528-533. Epub 2017/07/25.

Poesen K, De Schaepdryver M, Stubendorff B, et al. Neurofilament Markers for ALS Correlate with Extent of Upper and Lower Motor Neuron Disease. Neurology. 2017;88(24):2302-2309. Epub 2017/05/12.

Proudfoot M, Jones A, Talbot K, et al. The ALSFRS as an outcome measure in therapeutic trials and its relationship to symptom onset. Amyotroph Lateral Scler Frontotemporal Degener. 2016;17(5-6):414-25. Epub 2016/02/11.

Rabkin JG, Gordon PH, McElhiney M, et al. Modafinil treatment of fatigue in patients with ALS: a placebo-controlled study. Muscle Nerve. 2009;39(3):297-303.

Ramamoorthy D, Severson K, Ghosh S, et al. Identifying patterns in amyotrophic lateral sclerosis progression from sparse longitudinal data. Nature Computational Science. 2022;2(9):605-616.

Ramirez C, Piemonte ME, Callegaro D, et al. Fatigue in amyotrophic lateral sclerosis: frequency and associated factors. Amyotroph Lateral Scler. 2008;9(2):75-80.

Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and

Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24. Epub 2015/03/05.

Rigi M, Almarzouqi SJ, Morgan ML, et al. Papilledema: epidemiology, etiology, and clinical management. Eye Brain. 2015;7:47-57. Epub 20150817.

Robberecht W, Philips T. The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci. 2013;14(4):248-64. Epub 20130306.

Rosen DR. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993;364(6435):362.

Rossi D, Volanti P, Brambilla L, et al. CSF neurofilament proteins as diagnostic and prognostic biomarkers for amyotrophic lateral sclerosis. J Neurol. 2018;265(3):510-521. Epub 20180110.

Sanjak M, Salachas F, Frija-Orvoen E, et al. Quality control of vital capacity as a primary outcome measure during phase III therapeutic clinical trial in amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2010;11(4):383-8.

Shefner JM, Liu D, Leitner ML, et al. Quantitative strength testing in ALS clinical trials. Neurology. 2016;87(6):617-24. Epub 2016/07/06.

Skillbäck T, Mattsson N, Blennow K, et al. Cerebrospinal fluid neurofilament light concentration in motor neuron disease and frontotemporal dementia predicts survival. Amyotroph Lateral Scler Frontotemporal Degener. 2017;18(5-6):397-403. Epub 20170206.

Steinacker P, Huss A, Mayer B, et al. Diagnostic and prognostic significance of neurofilament light chain NF-L, but not progranulin and S100B, in the course of amyotrophic lateral sclerosis: Data from the German MND-net. Amyotroph Lateral Scler Frontotemporal Degener. 2017;18(1-2):112-119. Epub 20161105.

Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, et al. Targeting Huntingtin Expression in Patients with Huntington's Disease. N Engl J Med. 2019;380(24):2307-2316. Epub 2019/05/06.

Tattevin P, Tchamgoué S, Belem A, et al. Aseptic meningitis. Rev Neurol (Paris). 2019;175(7-8):475-480. Epub 20190730.

Thakore NJ, Drawert BJ, Lapin BR, et al. Progressive arm muscle weakness in ALS follows the same sequence regardless of onset site: use of TOMS, a novel analytic method to track limb strength. Amyotroph Lateral Scler Frontotemporal Degener. 2021;22(5-6):380-387. Epub 20210223.

Thompson AG, Gray E, Verber N, et al. Multicentre appraisal of amyotrophic lateral sclerosis biofluid biomarkers shows primacy of blood neurofilament light chain. Brain Commun. 2022;4(1):fcac029. Epub 20220209.

Thouvenot E, Demattei C, Lehmann S, et al. Serum neurofilament light chain at time of diagnosis is an independent prognostic factor of survival in amyotrophic lateral sclerosis. Eur J Neurol. 2020;27(2):251-257. Epub 20190918.

Traynor BJ, Zhang H, Shefner JM, et al. Functional outcome measures as clinical trial endpoints in ALS. Neurology. 2004;63(10):1933-5.

Vacchiano V, Mastrangelo A, Zenesini C, et al. Plasma and CSF Neurofilament Light Chain in Amyotrophic Lateral Sclerosis: A Cross-Sectional and Longitudinal Study. Front Aging Neurosci. 2021;13:753242. Epub 20211022.

Vågberg M, Norgren N, Dring A, et al. Levels and Age Dependency of Neurofilament Light and Glial Fibrillary Acidic Protein in Healthy Individuals and Their Relation to the Brain Parenchymal Fraction. PLoS One. 2015;10(8):e0135886. Epub 20150828.

Van den Berg L, Rothstein J, Shaw P, et al. Results From the Phase 1 Trial and Open-Label Extension Evaluating BIIB078 in Adults With C9orf72-ALS. Presented at the European Network for the Cure of Amyotrophic Lateral Sclerosis - 20th Meeting.

van Eijk RPA, de Jongh AD, Nikolakopoulos S, et al. An old friend who has overstayed their welcome: the ALSFRS-R total score as primary endpoint for ALS clinical trials. Amyotroph Lateral Scler Frontotemporal Degener. 2021;22(3-4):300-307. Epub 20210202.

Verde F, Silani V, Otto M. Neurochemical biomarkers in amyotrophic lateral sclerosis. Curr Opin Neurol. 2019;32(5):747-757.

Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. Orphanet J Rare Dis. 2009;4:3. Epub 2009/02/03.

Winer L, Srinivasan D, Chun S, et al. SOD1 in cerebral spinal fluid as a pharmacodynamic marker for antisense oligonucleotide therapy. JAMA Neurol. 2013;70(2):201-7.

Writing Group, Edaravone (MCI-186) ALS 19 Study Group. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2017;16(7):505-512. Epub 2017/05/15.

Xu L, Liu T, Liu L, et al. Global Variation in Prevalence and Incidence of Amyotrophic Lateral Sclerosis: a Systematic Review and Meta-analysis. J Neurol. 2020;267(4):944-953. Epub 2019/12/03.

Yuan A, Rao MV, Veeranna, et al. Neurofilaments and Neurofilament Proteins in Health and Disease. Cold Spring Harb Perspect Biol. 2017;9(4) Epub 20170403.

Zetterberg H, Jacobsson J, Rosengren L, et al. Cerebrospinal fluid neurofilament light levels in amyotrophic lateral sclerosis: impact of SOD1 genotype. Eur J Neurol. 2007;14(12):1329-33. Epub 20070926.

Zou ZY, Zhou ZR, Che CH, et al. Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2017;88(7):540-549. Epub 2017/01/05.

# 9. LISTING OF APPENDICES

# APPENDIX 1: STATISTICAL METHODOLOGY

## 9.1. Prespecified Efficacy Analyses in VALOR Reporting and Initial ISE

### 9.1.1. Prespecified Analyses in VALOR Final Reporting (VALOR SAP V2.0)

The analysis populations for VALOR final reporting are as follows:

- Primary efficacy analysis population denoted as FPS (mutation/slope): randomized and dosed participants who met the enrichment criteria for faster progression
- SPS (mutation/slope): randomized and dosed participants in the broader SOD1-ALS population who did not meet the enrichment criteria for fast progression
- ITT population: all participants randomized and dosed in VALOR
- Subgroup populations of FPS (NF-based) and SPS (NF-based): all participants randomized and dosed whose baseline Nf level was ≥ median baseline Nf and < median baseline Nf, respectively

The JRT was used for statistical inference of the primary endpoint, change from baseline to Week 28 in the ALSFRS-R total score in the FPS (mutation/slope). Table 20 lists the endpoints defined in VALOR and the method of analysis. The statistical testing in the FPS (mutation/slope) was conducted sequentially at a 2-sided alpha level of 0.05 to control the overall Type I error; if statistical significance was not achieved for an endpoint in the hierarchy, all endpoints of a lower rank were exploratory.

**Table 20: Endpoints Analyzed in VALOR**

| Endpoint Statistical Testing | FPS (Mutation/Slope) Population/ (Analysis Method) | SPS (Mutation/Slope) Population |
|---|---|---|
| **Primary (Formal Statistical Testing)** | Change from baseline to Week 28 in ALSFRS-R total score / (JRT+MI) | Change from baseline (ratio) to Week 28 in total CSF SOD1 protein/(ANCOVA+MI) |
| **Secondary (Formal Statistical Testing)** | Secondary endpoints were to be tested using a sequential closed testing procedure in order of the following ranking:<br>1) change from baseline (ratio) to Week 28 in total CSF SOD1 protein / (ANCOVA+MI);<br>2) change from baseline (ratio) to Week 28 in plasma NfL / (ANCOVA+MI);<br>3) change from baseline to Week 28 in percent-predicted SVC / (JRT+MI);<br>4) change from baseline to Week 28 in HHD megascore / (ANCOVA+MI);<br>5) time to death or PV / (stratified log-rank);<br>6) time to death / (stratified log-rank) | Not applicable |
| **Exploratory (No Formal Testing; Nominal P-Values)** | Changes from baseline in QoL measures (ALSAQ-5, FSS, EQ-5D-5L) and other biomarkers (CSF NfL, CSF and plasma pNfH)/ (ANCOVA+MI) | Changes from baseline in clinical function (ALSFRS-R, SVC, HHD), biomarkers, QoL measures (ANCOVA+MI);<br>time to death or PV, time to death (stratified log-rank) |

NOTE 1: JRT + MI = Joint rank in conjunction with multiple imputation to handle missing data after withdrawal from study for reasons other than death; ANCOVA + MI = Analysis of covariance in conjunction with multiple imputation to handle missing data after withdrawal from study.
NOTE 2: ANCOVA for ranked scores and ANCOVA for change from baseline includes trial group as a fixed effect and adjusts for covariates (baseline disease duration since symptom onset, corresponding baseline value for the endpoint, and use of riluzole or edaravone). MI model includes trial group, use of riluzole or edaravone, and the corresponding baseline value for the endpoint.

The JRT accounts for both functional decline and survival and allows for a statistical test of the treatment effect while accounting for truncation of data owing to deaths. In VALOR, the JRT combined ALSFRS-R total score and time to death into a composite score, with death treated as worst outcome. The change in each participant's ALSFRS-R score from baseline to Week 28 was compared with that of every other participant in the trial. Participants who died were assigned progressively lower ranks based on their time of death. The ranked scores were assessed with the use of ANCOVA. Joint rank analysis was performed in conjunction with MI to account for missing data due to withdrawals not accounted for by death. The estimated between-group difference was obtained from the ANCOVA model for change from baseline to Week 28 in the ALSFRS-R score in conjunction with MI to handle missing data after withdrawal from the study. See Table 20 for analysis methods of all endpoints in the FPS and SPS (mutation/slope).

Kaplan-Meier analyses were conducted for time to event endpoints and tested using a log-rank test, stratified by trial group and use of riluzole or edaravone. Participants who did not meet the endpoint definition were censored on the latest of last contact date or date of withdrawal in

VALOR. A Cox proportional hazards model was also conducted and included trial group with adjustment for covariates (baseline disease duration since symptom onset, baseline ALSFRS-R total score, and use of riluzole or edaravone).

Descriptive analyses were presented for clinical function, biomarker, QoL, and time to event endpoints in the ITT population; a nominal p-value was presented for total CSF SOD1 protein.

Exploratory analyses in FPS and SPS (NF-based) subgroups based on the median baseline plasma NfL value were also prospectively planned for the primary and secondary endpoints. Time to event endpoints were analyzed using a log-rank test stratified by the median baseline plasma NfL (< median, ≥ median). Recognizing that adjusting for neurofilament as a continuous covariate may more accurately control for disease heterogeneity at Baseline, sensitivity analyses for change from baseline to Week 28 in ALSFRS-R total score were also prespecified in the FPS and SPS (mutation/slope) populations with adjustment for baseline plasma NfL as a covariate.

### 9.1.2. Prespecified Analyses (ISE SAP V2.0): July 2021 Data Cutoff for OLE

At the time of randomization in VALOR, the treatment sequence (early-start and delayed-start) for the integrated analyses of VALOR and OLE was predetermined and represents the systematic difference between the 2 treatment arms.

An integrated efficacy analysis was performed by integrating final data from VALOR and interim data for OLE from an initial data cutoff on 16 July 2021. The analyses were based on the ISE SAP V2.0. Participants in the "early-start" group (those who initiated tofersen in VALOR) were compared with participants in the placebo/delayed-start group (those who had the opportunity to receive tofersen in the OLE after 28 weeks on placebo). Baseline was VALOR Day 1. The analyses were similar to those specified for VALOR except for including follow-up data from OLE and were based on disease progression subgroups (FPS and SPS, mutation/slope and NF-based) in clinical function, biomarker, time to event and QoL endpoints. There was no formal statistical testing.

## 9.2. Prespecified Analyses (ISE SAP V3.0): January 2022 Data Cutoff for OLE

Following the learnings from VALOR and ISE analyses based on the July 2021 data cutoff around neurofilament as a prognostic factor and recognizing that adjusting for a continuous variable as a covariate more precisely controls for individual heterogeneity than dichotomizing the population into categorical subgroups, modified analyses were prospectively planned in the ITT population for all clinical function and QoL endpoints in the ITT population with adjustment for baseline plasma NfL as a continuous covariate in the ANCOVA + MI analysis instead of disease duration. Kaplan-Meier plots, log-rank test (stratified by median baseline plasma NfL), and Cox proportional hazards model with adjustment for baseline plasma NfL were prespecified for time to event endpoints. These modified analyses were conducted at Week 52 of the integrated data from VALOR and OLE. Following the ITT principle, all 108 participants were included. There was no adjustment for testing multiple endpoints; nominal p-values are presented.

Supportive analyses in disease progression subgroups (as defined by mutation/slope and NF-based) adjusting for baseline NfL as a covariate are also provided. Two additional survival

endpoints included time to death incorporating postwithdrawal vital status, and time to death, PV or withdrawal due to disease progression. Postwithdrawal vital status data were collected for participants to the time of the data cutoff in January 2022. Biogen team members who worked on ISE SAP V3.0 were firewalled from ongoing OLE data.

## 9.3. Modified and Exploratory Analyses

Modified analyses for VALOR incorporating baseline plasma NfL instead of disease duration as a covariate in the ANCOVA model were also performed in 1) secondary efficacy endpoints in disease progression subgroups (mutation/slope and NF-based) and 2) the full ITT population for the primary, secondary, and exploratory endpoints. Similarly modified analyses were conducted for the integrated VALOR+OLE data based on the 16 July 2021 data cutoff for 1) all clinical function and QoL endpoints in the ITT population with adjustment for baseline plasma NfL as a covariate in the ANCOVA analysis as well as 2) subgroup analyses by median plasma NfL for all other clinical function and QoL endpoints (ALSFRS-R was prespecified). Results from this set of data are not presented in this document.

An alternative method of implementing the JRT, denoted by JRT*, has been proposed. JRT* is also used in conjunction with MI to handle missing data due to withdrawals for reasons other than death. The difference with JRT is that the response variable is the change in ranks pre- and postbaseline instead of the ranked score for change from baseline, and the ranked scores of continuous baseline variables are used as covariates. The JRT*+MI analyses were conducted at both Week 28 for VALOR and Week 52 for the integrated VALOR+OLE based on the 16 January 2022 data cutoff.

To demonstrate the impact of accounting for intercurrent events, JRT in conjunction with MI was performed on ALSFRS-R data at Week 52 in the ITT population for the integrated dataset as well as JRT*+MI. Intercurrent events are listed below, with details of how they were handled using JRT for ALSFRS-R total score at Week 28 for VALOR and at Week 52 for the integrated dataset based on the 16 January 2022 data cutoff. All these analyses were conducted post-hoc after the data cutoff in January 2022 and are based on the ITT population adjusting for baseline NfL as a covariate.

- **Deaths and withdrawals**: deaths are treated as the worst outcome and ranked the lowest based on time to death; all other withdrawals use the imputed score from MI.
- **Deaths, withdrawals, and PV**: deaths are treated as the worst outcome and ranked the lowest based on time to death; participants with PV are ranked higher than deaths but lower than all other participants using their time to reaching first day of PV (protocol-defined criteria); all other withdrawals use the imputed score from MI.
- **Deaths, PV, and withdrawals due to disease progression**: deaths are treated as the worst outcome and ranked the lowest based on time to death; participants with PV are ranked higher than deaths but lower than all other participants using their time to reaching first day of PV (protocol-defined criteria); withdrawals due to disease progression are ranked higher than deaths and PV but lower than completers and other withdrawals based on time to withdrawal; all other withdrawals use the imputed score from MI.

These results investigating the JRT*+MI and various intercurrent events are presented in Appendix 2, Table 22.

To further investigate adjustment of covariates across different populations and different methods, analyses for changes from baseline to Weeks 28 and Week 52 in ALSFRS-R total score were conducted in different populations (ITT, FPS and SPS, mutation/slope and NF-based) with adjustment for different covariates using 1) the ANCOVA+MI, 2) the JRT+MI method used in the primary efficacy analysis, and 3) JRT*+MI method. These results are presented in Appendix 2, Figure 25 and Figure 28.

Following the VALOR readout in recognition of the importance of neurofilament reductions, a statistical model with a causal inference component was developed to formally interrogate the relationship on an individual basis. This model assesses the relationship between early tofersen-driven reductions in plasma NfL at Week 16 and slowing of clinical progression over time. A similar model was also developed to investigate the relationship between tofersen-induced reductions in neurofilament and survival outcomes.

## 9.4. Statistical Methods for Integrated Safety Data from Study 101 and the OLE

Data from the randomized controlled period of Study 101 were integrated with the OLE to inform the longer-term benefit/risk of tofersen. This integrated analysis was first performed based on final data for Study 101 and the 16 July 2021 interim data cutoff of the OLE, concurrent with the completion of VALOR, at which time all VALOR participants had the opportunity for at least 6 months of follow-up. A second and third integrated analysis were performed based on the 16 January 2022 and 15 July 2022 interim data cutoffs of the OLE, respectively, at which time all VALOR participants had the opportunity for at least 12 and 18 months of follow-up. Integrated safety data were analyzed and are presented in this summary with focus on the 15 July 2022 data cutoff.

The focus of this summary is on participants from VALOR and the long-term safety of participants who received tofersen in Study 101 or the OLE.

All safety outputs were analyzed for the safety population, i.e., all randomized and dosed participants. In the integrated analyses, the focus is on the tofersen-treated period; for participants who received placebo during Study 101, data are not presented from the placebo-treated period and baseline is defined as the first dose of tofersen. The only safety data from placebo presented as part of the integrated analyses are from VALOR (RC2) to serve as a reference. A subset of safety analyses is presented by disease progression subgroup (i.e., “enriched” and “other”) in which the “enriched” subgroup consists of participants who met prognostic enrichment criteria for rapid disease based on prerandomization ALSFRS-R slope and mutation. “Other” subgroup consists of all other eligible participants enrolled.

The pooling strategy used for the safety analyses is provided in Table 21.

## Table 21: Tofersen Pooling Strategy for Safety

| | 233AS101 Part C (VALOR participants) | | VALOR and OLE (VALOR participants) | Study 101 Part A and B and OLE (Part A and B participants) [a] | Overall Study 101 and OLE (Parts A, B, and C participants) | |
|---|---|---|---|---|---|---|
| **Treated Periods** | Placebo-controlled period (RC) | | Tofersen-treated period | Tofersen-treated period | Tofersen-treated period (ABCL) | |
| **Pooled Groups[b]** | Pool RC1 | Pool RC2 | Pool CL | Pool ABL | Pool ABCL1 | Pool ABCL2 |
| **Treatment Cohorts** | Tofersen 100 mg | Placebo | tofersen 100 mg: VALOR and the OLE | Total tofersen 100 mg: Study101 Part A and B and the OLE | Total tofersen 100 mg | Total tofersen all doses |
| **Participants Included** | For those who received tofersen in VALOR during the randomized period of the study. | For those who received placebo in VALOR during the randomized period of the study. | Total of all participants in VALOR exposed to tofersen 100 mg during either VALOR (i.e., CL1) or initiated during the OLE (CL2); regardless of whether they entered the OLE. Note a subset of tables will be presented for CL1 and CL2. | All participants from Part A or Part B of 101 who received tofersen 100 mg in either 101 Part B or in 102. | All participants who received tofersen 100 mg in either 101 Part B or VALOR or in the OLE. | Total of all participants who were exposed to tofersen. Participants who did not continue into the OLE will also be included. Participants who only ever received doses <100 mg will also be included. |
| **Data Included** | All data from tofersen 100 mg during VALOR. | All data from placebo during VALOR. | All data from tofersen 100 mg during VALOR and the OLE. For those who initiated tofersen in the OLE (CL2), data from the placebo-controlled period in VALOR are not included here. | For participants from Part A or Part B who started on a lower dose in the OLE, this will include data from first dose of 100 mg in the OLE For participants from Part B who started tofersen 100 mg, all data will be included. | For participants from Part A or B who started on a lower dose in the OLE, this includes data from first dose of 100 mg in the OLE. For VALOR participants this is data included under Pool CL. | For participants from VALOR this includes data from CL. For participants from Part A or Part B of Study 101 this includes all data from first dose of tofersen. Data from all doses of tofersen are included. |

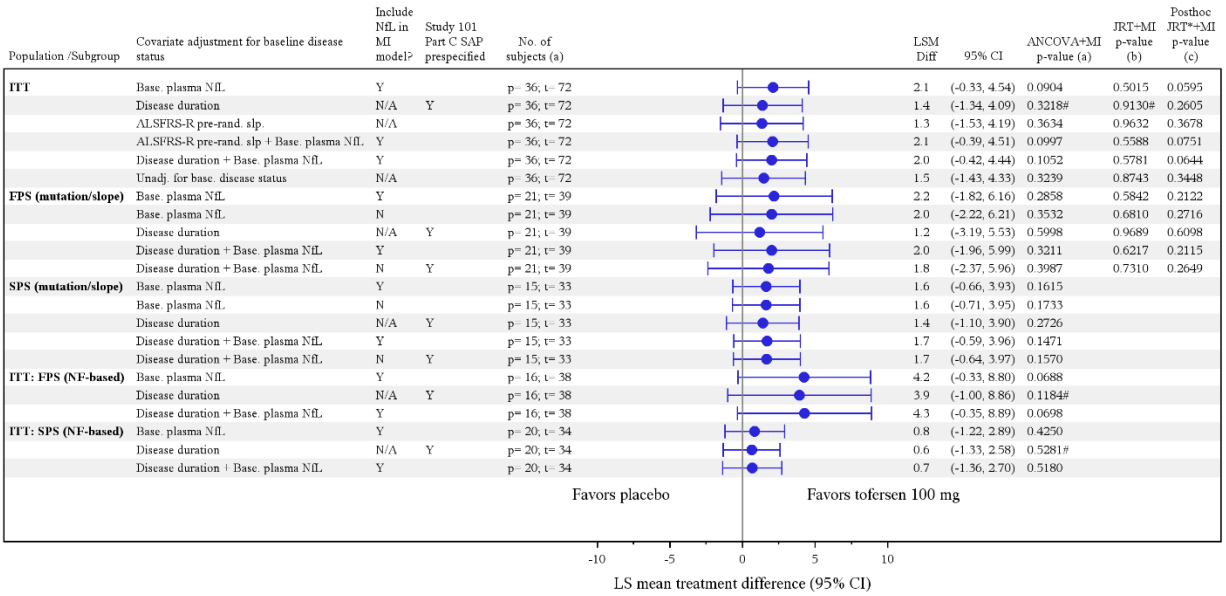
[a] ABL only applies to some tables.

[b] RC: 233AS101 Part C randomized controlled period; CL: 233AS101 Part C and long-term extension (233AS102); ABCL: 233AS101 Part A/Part B/Part C and long-term extension (233AS102).

# APPENDIX 2: ADDITIONAL SUPPORTIVE ANALYSES

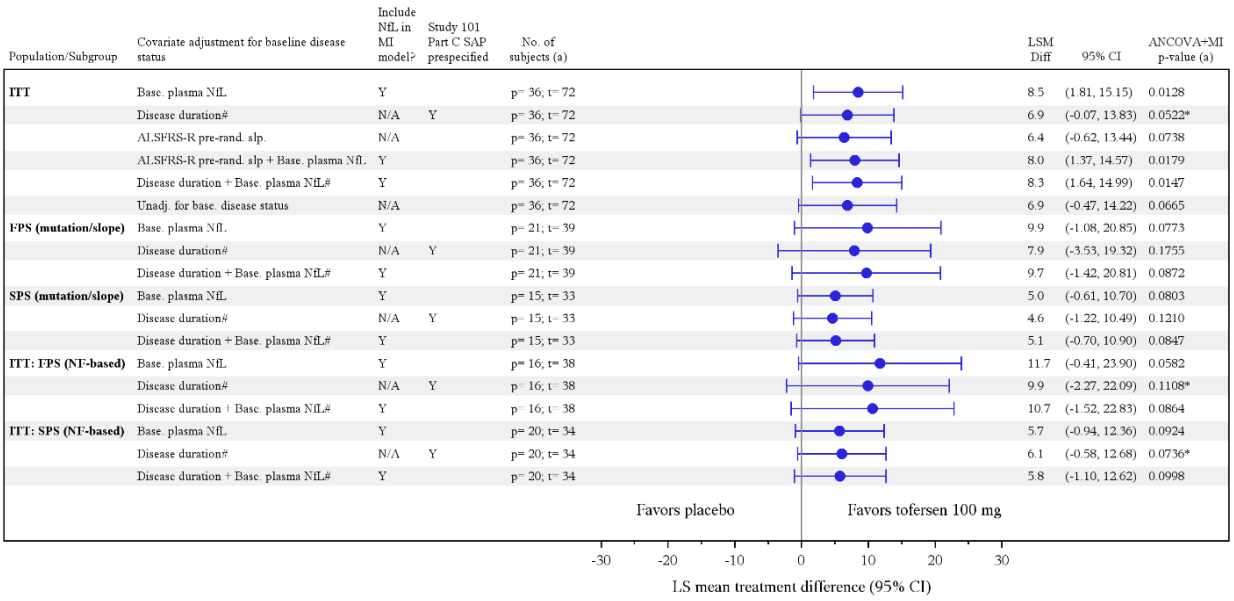
The forest plots in Figure 25, Figure 26, Figure 27, and Figure 28 for ALSFRS-R, SVC, and HHD megascore demonstrate that the trends are consistently in favor of tofersen across different populations and disease progression subgroups (FPS and SPS, mutation/slope and NF-based) regardless of which covariates are adjusted for. The results from JRT*+MI are generally consistent with those of ANCOVA+MI. The findings are also consistent for Week 28 and Week 52 analyses.

## Figure 25: VALOR: Forest Plot of ALSFRS-R Total Score Change from Baseline to Week 28



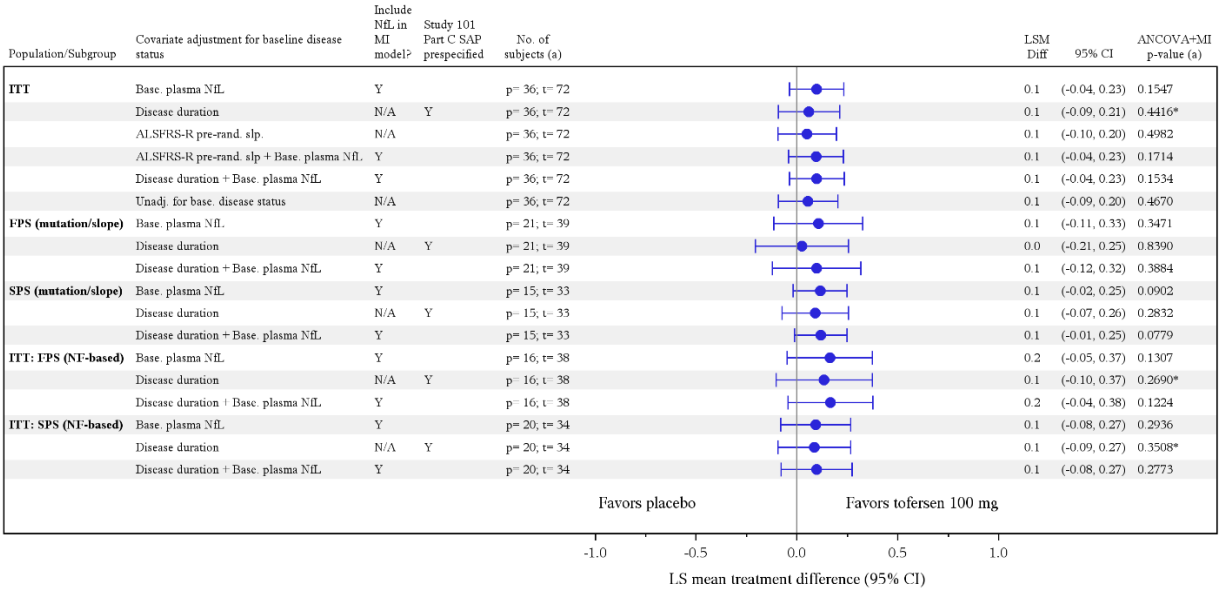

NOTE 1: For the analyses with 'Include NfL in MI model?' = Y, multiple imputation including use of riluzole or edaravone, baseline plasma NfL, and the relevant baseline and postbaseline values for the endpoint is used for missing data. For the analyses with 'Include NfL in MI model?' = N or N /A, multiple imputation including use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data. The multiple imputation is run separately for each population (FPS, SPS, ITT) except for the SAP prespecified ITT analysis adjusting for disease duration where the combined MI dataset from the FPS and SPS population is used.
NOTE 2: The ANCOVA models include treatment as a fixed effect and adjusting for the following covariates: the specified covariate(s), baseline ALSFRS-R, and use of riluzole or edaravone.
NOTE 3: FPS and SPS (mutation/slope) are disease progression subgroups based on mutation type and prerandomization slope as defined in the protocol.
NOTE 4: FPS and SPS (NF-based) are disease progression subgroups defined by the median baseline plasma NfL (>= and < Median (75.60 pg/mL)).
# No statistical testing was prespecified for ITT population in 233AS101 SAP; all p-values are post-hoc for ITT prespecified 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 in pre- and post- treatment ranked scores; deaths are ranked the lowest; MI is used for handling missing data due to withdrawals other than death. Continuous covariates are included in the ANCOVA as ranked scores.
p = placebo; t = tofersen 100 mg.

## Figure 26: VALOR: Forest Plot of Percent-Predicted SVC Change from Baseline to Week 28



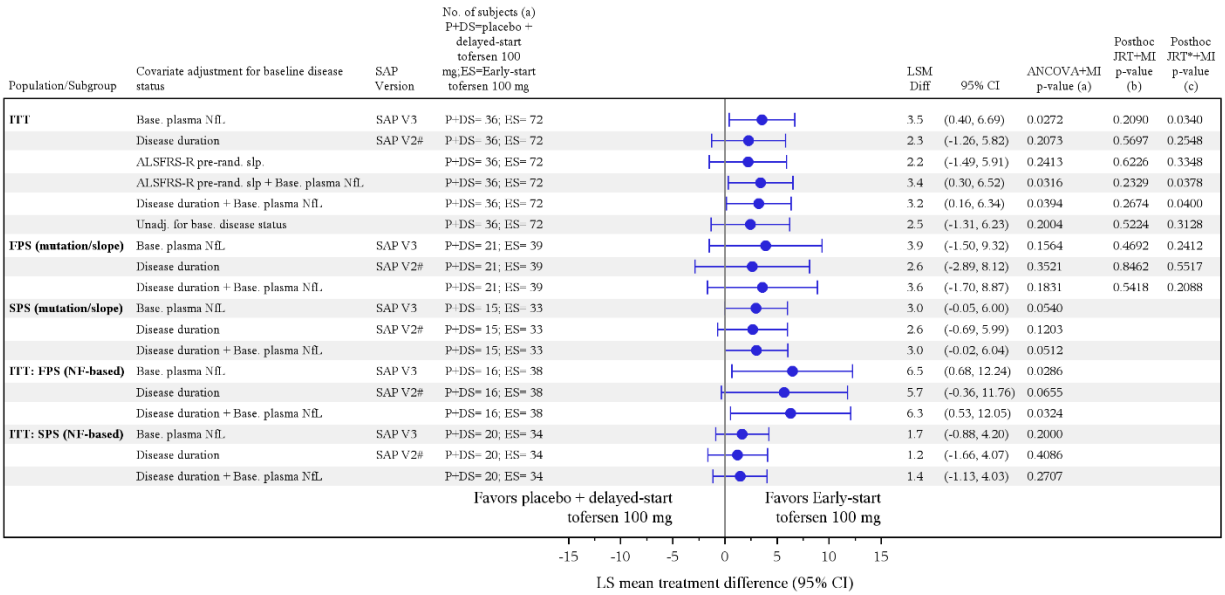

NOTE 1: For the analyses with 'Include NfL in MI model?' = Y, multiple imputation including use of riluzole or edaravone, baseline plasma NfL, and the relevant baseline and postbaseline values for the endpoint is used for missing data. For the analyses with 'Include NfL in MI model?' = N or N/A, multiple imputation including use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data. The multiple imputation is run separately for each population (FPS, SPS, ITT) except for the SAP prespecified ITT analysis adjusting for disease duration where the combined MI dataset from the FPS and SPS population is used.
NOTE 2: The ANCOVA models include treatment as a fixed effect and adjusting for the following covariates: the specified covariate(s), baseline percent-predicted SVC, and use of riluzole or edaravone.
NOTE 3: FPS and SPS (mutation/slope) are disease progression subgroups based on mutation type and prerandomization slope as defined in the protocol.
NOTE 4: FPS and SPS (NF-based) are disease progression subgroups defined by the median baseline plasma NfL (>= and < Median (75.60 pg/mL)).
#The baseline ALSFRS-R is included as an additional covariate in ANCOVA.
* No statistical testing was prespecified for ITT population in 233AS101 SAP; all p-values are post-hoc for ITT prespecified analyses.
(a) From the listed ANCOVA analysis based on change from baseline.
p = placebo; t = tofersen 100 mg.

## Figure 27: VALOR: Forest Plot of HHD Megascore Change from Baseline to Week 28




NOTE 1: For the analyses with 'Include NfL in MI model?' = Y, multiple imputation including use of riluzole or edaravone, baseline plasma NfL, and the relevant baseline and postbaseline values for the endpoint is used for missing data. For the analyses with 'Include NfL in MI model?' = N or N/A, multiple imputation including use of riluzole or edaravone, and the relevant baseline and postbaseline values for the endpoint is used for missing data. The multiple imputation is run separately for each population (FPS , SPS , ITT) except for the SAP prespecified ITT analysis adjusting for disease duration where the combined MI dataset from the FPS and SPS population is used.
NOTE 2: The ANCOVA models include treatment as a fixed effect and adjusting for the following covariates: the specified covariate(s), baseline HHD overall megascore, and use of riluzole or edaravone.
NOTE 3: FPS and SPS (mutation/slope) are disease progression subgroups based on mutation type and prerandomization slope as defined in the protocol.
NOTE 4: FPS and SPS (NF-based) are disease progression subgroups defined by the median baseline plasma NfL (≥ and < median (75.60 pg/mL)).
* No statistical testing was prespecified for ITT population in 233AS101 SAP; all p-values are post-hoc for ITT prespecified analyses.
(a) From the listed ANCOVA analysis based on change from baseline.
p = placebo; t = tofersen 100 mg.

## Figure 28: Forest Plot of ALSFRS-R Total Score Change from VALOR Baseline to Week 52 (VALOR + OLE)




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 cutoff; 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 cutoff.
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 FPS (mutation/slope) and SPS (mutation/slope) 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 FPS (mutation/slope) and SPS (mutation/slope) 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.
NOTE 4: FPS and SPS (mutation/slope) are disease progression subgroups based on mutation type and prerandomization slope as defined in the protocol.
NOTE 5: FPS and SPS (NF-based) are disease progression subgroups defined by the median baseline plasma NfL (>= and < Median (75.60 pg/mL)).
# No statistical testing was prespecified in SAP V2.0; all p-values are post-hoc for SAP V2.0 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.
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; FPS = fast progression subgroup; SPS = slow progression subgroup.

Handling of Intercurrent Events Using Joint Rank Methodology

Table 22 summarizes the p-values obtained from each of the analyses conducted in addition to the p-value obtained from the ANCOVA for change from baseline in conjunction with MI. The results from JRT*+MI are consistent with those from ANCOVA+MI. At 52 weeks from VALOR baseline, there is nominal statistical significance on JRT* incorporating different intercurrent events, and the p-values are more aligned with the ANCOVA+MI p-values.

**Table 22: Summary of p-Values for Change in ALSFRS-R Total Score From VALOR Baseline (ITT population; adjusted for baseline plasma NfL)**

| Statistical Methodology | Week 28 (Tofersen vs. Placebo) | Week 52[a] (Early-Start vs. Delayed-Start) |
|---|---|---|
| **ANCOVA + MI** | p = 0.0904 | p = 0.0272 |
| **JRT*+MI Incorporating Deaths** | p = 0.0595 | p = 0.0340 |
| **JRT*+MI Incorporating Deaths + PV** | p = 0.0635 | p = 0.0394 |
| **JRT*+MI Incorporating Deaths + PV + Withdrawal Due To Disease Progression** | p = 0.0595 | p = 0.0267 |
| **JRT+MI Incorporating Deaths** | p = 0.5015 | p = 0.2090 |
| **JRT+MI Incorporating Deaths + PV** | p = 0.5694 | p = 0.2640 |
| **JRT+MI Incorporating Deaths + PV + Withdrawal Due to Disease Progression** | p = 0.5397 | p = 0.2412 |

[a] Based on integrated data from VALOR and OLE. Interim data cutoff for OLE (data cutoff date: 16 January 2022)
NOTE 1: All analyses are post-hoc, with the exception of the ANCOVA+MI integrated data analysis for VALOR and OLE based on January 2022 data cutoff which was prespecified in ISE SAP V3.0. 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 postbaseline values.
NOTE 3: The JRT is based on the ANCOVA for ranked score for the change from baseline and includes original scale values for continuous covariates; JRT* 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.