FDA Briefing Document

NDA # 215887

Drug name: tofersen

Applicant: Biogen

Peripheral and Central Nervous System Drugs Advisory Committee (PCNS) Meeting

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Division of Neurology 1/Office of Neuroscience

Center for Drug Evaluation and Research

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Glossary

AC	Advisory Committee
AE	adverse event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale - Revised
ASO	antisense oligonucleotide
BD	Briefing Document
BRF	Benefit-Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDTL	Cross-Discipline Team Leader
CFB	change from baseline
CI	confidence interval
CSF	cerebrospinal fluid
FDA	Food and Drug Administration
HHD	handheld dynamometry
IA	integrated assessment
IND	Investigational New Drug Application
ISE	Integrated Summary of Efficacy
IT	intrathecal
ITT	intent-to-treat population
MI	multiple imputation
mITT	modified intent-to-treat population
NfL	neurofilament light chain
Nfs	neurofilament proteins
OLE	open-label extension
PD	pharmacodynamics
PV	permanent ventilation
REMS	risk evaluation and mitigation strategy
RPM	Regulatory Project Manager
SAE	serious adverse event

SAP	Statistical Analysis Plan
SD	standard deviation
SOD1	superoxide dismutase
SVC	slow vital capacity

1 Executive Summary/Draft Points for Consideration by the Advisory Committee

1.1 Purpose/Objective of the AC Meeting

The Food and Drug Administration (FDA) is convening this Advisory Committee (AC) meeting to discuss whether the observed reduction of neurofilament light chain (NfL) is reasonably likely to predict clinical benefit and support accelerated approval of tofersen for the treatment of patients with Amyotrophic Lateral Sclerosis (ALS) associated with mutations in the superoxide dismutase 1 (SOD1) gene (SOD1-ALS), taking into account the severity and very low prevalence of SOD1-ALS and the significant unmet need for effective treatments for this life-threatening disease. The AC will also discuss whether clinical data from the placebo-controlled study and available long-term extension study results, with additional supporting results from the effects on relevant biomarkers (i.e., changes in NfL and/or reductions in SOD1), provide convincing evidence of the effectiveness of tofersen in the treatment of patients with SOD1-ALS.

1.2 Context for Issues to Be Discussed at the AC

ALS is a rapidly progressive and fatal neurodegenerative disease that primarily affects motor neurons in the cerebral motor cortex, brainstem, and spinal cord, leading to loss of voluntary movement and development of difficulty in swallowing, speaking, and breathing, ultimately leading to death. ALS may also cause cognitive and behavioral changes. Most cases of ALS are sporadic. Five to ten percent of ALS cases are familial and are associated with approximately 30 different genes. The superoxide dismutase 1 (SOD1) gene is associated with 20% of familial cases and approximately 2% of sporadic ALS cases. The prevalence of SOD1-ALS in the US is estimated to be less than 500 cases. The mechanism by which mutations in the SOD1 gene cause ALS are not fully understood; however, gain-of-function mutations in SOD1 are thought to cause formation and accumulation of toxic SOD1 protein aggregates.[1]. There are over 200 causative SOD1 mutations associated with ALS that have been identified to date.

Similar to sporadic ALS, SOD1-ALS patients can present with weakness and muscle atrophy in different areas of the body, with about 75 percent of patients first experiencing weakness in their limbs, and about 25 percent presenting with difficulty swallowing and/or speaking (i.e., bulbar-onset ALS). Respiratory muscles are also affected, leading to respiratory failure and death of most patients within 3 to 5 years from the onset of symptoms. Approximately 10 percent of ALS patients survive for 10 or more years. Shorter survival may be associated with older age at onset, bulbar-onset disease, and faster rate of respiratory dysfunction. In general, SOD-1 ALS has similar clinical characteristics to sporadic ALS, with a combination of upper and lower motor neuron involvement. However, the degree of upper versus lower motor neuron involvement, age of onset, and rate of progression can vary greatly as a function of the specific SOD1 pathogenic variant.

There are three FDA-approved treatments for ALS: riluzole, edaravone, and sodium phenylbutyrate/taurursodiol.

[1] Abati, Bresolin, Comi, & Corti. Expert Opin Ther Targets. 2020 Apr;24(4):295-310.

Despite current treatment options, most patients with SOD1-ALS continue to progress rapidly, with significant muscle weakness, which ultimately leads to respiratory failure and death. There remains a significant unmet clinical need for effective treatments for patients living with ALS. There are no approved therapies that are targeted to the SOD1 mutation in ALS.

1.3 Brief Description of Issues for Discussion at the AC

Tofersen is an antisense oligonucleotide (ASO) designed to bind and degrade SOD1 mRNA to reduce synthesis of SOD1 protein. A toxic form of SOD1 protein is implicated in the pathophysiology of SOD1-ALS. Tofersen targets the untranslated region of the mRNA for human SOD1 to reduce the amount of SOD1 protein synthesis and accumulation. Tofersen reduces SOD1 protein translation, an event that is upstream of the pathological mechanisms implicated in SOD1-ALS. It is therefore anticipated that any therapeutic benefit of tofersen would apply to all SOD1-ALS patients, regardless of the mutation type.

Tofersen is administered by an intrathecal route of administration.

A single 28-week randomized, double-blind, placebo-controlled pivotal study was conducted in 108 adult patients with SOD1-ALS. Patients were randomized 2:1 to tofersen or placebo. Randomization was stratified by "fast progressor" and "non-fast progressor." Fast progressors were defined by genetic mutation and pre-randomization slope on the ALSFRS-R, and this fast progressor group formed the primary analysis population. The primary endpoint evaluated clinical function with the ALS Functional Rating Scale-Revised (ALSFRS-R) total score, and secondary endpoints evaluated respiratory function as measured by slow vital capacity (SVC), quantitative strength measurement through handheld dynamometry (HHD), and time to death or permanent ventilation. This study failed to show a statistically significant difference between the tofersen and placebo groups for the primary or secondary endpoints in the prespecified primary analysis in the fast progressor population. The estimated treatment effect for change from baseline in ALSFRS-R at week 28 in the primary analysis population was 1.2, 95% CI: -3.2, 5.5, with p=0.97 using the pre-specified primary joint rank analysis or p=0.60 using a prespecified supportive ANCOVA analysis.

Additional assessments in the study included biomarkers such as SOD1 protein in cerebrospinal fluid (CSF) concentrations and neurofilament light chain (NfL) in plasma. NfL is a marker of neuroaxonal damage. Most recently, scientific literature has established NfL as a biomarker that is significantly elevated in patients with ALS, even more so than in many other neurodegenerative diseases, and NfL levels have been shown to be prognostic for disease progression in ALS.[2,3]

A reduction in total CSF SOD 1 protein was observed at Week 28 in the tofersen group compared to the placebo group (38% difference in geometric means ratio for tofersen to placebo, nominal $p < 0.0001$). A 55% reduction in plasma NfL was observed at Week 28 in the tofersen group compared to a mean 12%

[2] 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. doi:10.1111/nan.12511

[3] 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. Published 2021 Jan 12. doi:10.1038/s41598-020-80370-6

increase in NfL observed in the placebo group (67% difference in geometric mean ratios for tofersen to placebo, nominal $p < 0.0001$).

The literature on NfL in ALS has evolved since the design and initiation of the pivotal study for tofersen. The reduction in SOD1 protein suggests target engagement. Post-hoc analysis suggest a correlation between the reduction in NfL in the treatment group with change from baseline in ALSFRS-R. Additional analyses by the Applicant, including a causal inference analysis, suggest that reduction in plasma NfL is associated with reduction in the decline of ALSFRS-R total score at Week 28. There are statistical uncertainties in these analyses; however, the data do not show apparent deviations from the model assumptions, and thus may support the predictive value of plasma NfL.

Following completion of the placebo-controlled study, all participants had the opportunity to enroll in an open-label extension (OLE) study, where they received open-label tofersen treatment but remained blinded to the treatment received in the double-blind study. The primary objective of the extension study was to evaluate safety and tolerability, but it also provides additional biomarker and clinical endpoint data through Week 52. After switching to tofersen in the open-label extension, patients previously receiving placebo experienced a similar reduction in NfL (44% from baseline of Study 102) after 24 weeks of treatment in the open-label period, followed by an apparent reduction in decline in ALSFRS-R total score at Week 52. Analyses based on the pre-specified methods of ALSFRS-R and other secondary clinical endpoints in the OLE showed favorable trends for tofersen, although the results were not nominally statistically significant, and some results were inconsistent (e.g., estimated hazard ratios for time to death were in opposite directions in the mITT and ITT populations). The Applicant also carried out additional post hoc, exploratory analyses of treatment benefit in the OLE, and nominally significant improvements were noted in both the ALSFRS-R and survival for patients originally randomized to tofersen compared to patients originally randomized to placebo. Based on the observed data of ALSFRS-R total score, the early-start tofersen group has shown numerically less decline in ALSFRS-R total score as compared to delayed-start group, which is consistent from Week 28 to Week 52. The consistent separation on ALSFRS-R between the two groups from Week 8 and onwards appears to further support the potential treatment effect of tofersen. However, these exploratory OLE analyses have limitations (e.g., post hoc choice of covariate and time points, multiplicity issues) that make interpretation of the results challenging.

The primary safety issues identified in the tofersen development program to date is the potential risk of serious neurologic adverse events that appear to be associated with the intrathecal route of administration of tofersen (i.e., myelitis, radiculitis, aseptic meningitis, papilledema, and elevated intracranial pressure). Aseptic meningitis, hydrocephalus, and inflammatory reactions in the peripheral and central nervous system have been observed in other ASO development programs for neurologic diseases with an intrathecal route of administration.

This is a situation where there is a negative clinical study that failed to show a statistically significant treatment effect in the prespecified primary analysis population. The study, as designed, was markedly underpowered and thus limited in its ability to detect a difference if there was a drug effect; however, there are available data from that study that indicate target engagement of the therapy and a reduction in a biomarker that has been shown to be correlated with disease progression and prognosis in patients

with ALS. There are also post hoc exploratory analyses of an open-label extension study that may be suggestive of a clinical benefit with a longer duration of treatment.

Accelerated approval is a particular type of approval that FDA may grant for 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 not itself a direct measure of the clinical benefit of interest but is instead reasonably likely to predict that clinical benefit. A surrogate endpoint is a marker, such as a laboratory measure, radiographic image, or other measure that is thought to predict clinical benefit but is not itself a measure of clinical benefit. To consider a drug for accelerated approval, a drug must demonstrate an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit; studies to demonstrate such an effect must be "adequate and well controlled." If approved under accelerated approval, additional studies may be required to confirm the anticipated clinical benefit.

The Applicant proposes the use of plasma NfL as a reasonably likely surrogate endpoint for the accelerated approval of tofersen in SOD1-ALS. The suitability of NfL as a surrogate endpoint in SOD1-ALS is being evaluated based on the following aspects:

- Mechanistic evidence that tofersen reduces SOD1 protein, the intended target of the drug, and a known contributor to the pathophysiology in patients with SOD1-ALS.
- Observed reduction in NfL, a biomarker of neurodegeneration that is known to be substantially elevated in patients with ALS and proportional to axonal damage.
- Scientific evidence demonstrating the prognostic value of plasma NfL in predicting disease progression and survival in ALS.
- An observed correlation between reduction in NfL and a reduction of decline on clinical outcomes such as the ALSFRS-R, including support from a causal inference model.

We are seeking the input of the committee to discuss whether the available evidence supports the use of NfL as a biomarker reasonably likely to predict clinical benefit in patients with SOD1-ALS.

Should we decide to approve tofersen under accelerated approval, additional confirmatory evidence of clinical benefit would be required to be demonstrated. Given the extremely rare nature of SOD1-ALS, and the very small pool of patients available for enrollment into a clinical study, a second adequate and well-controlled, double-blind, placebo-controlled study in symptomatic patients with SOD1-ALS does not appear to be feasible at this time. The data to confirm benefit may come from one of two sources. The Applicant has an ongoing double-blind, placebo-controlled study (Study 233AS303) in patients who are presymptomatic carriers of the SOD1 mutation. The study will evaluate if administration of tofersen can delay symptom onset in patients who have evidence of disease activity based on an increase in NfL levels to a prespecified threshold, compared to patients who receive placebo. This study is ongoing, but given the nature of the study, it may take several years to complete. The Applicant anticipates study results to be available in 2027.

Additionally, the Applicant plans to leverage results of the ongoing OLE study, in which all patients are continuing to receive treatment and remain blinded to their original treatment assignment in the double-blind study. The Applicant proposes to continue to follow these patients and assess survival. Analyses of these data will compare early-start tofersen patients, i.e., those who received tofersen in

the double-blind study, to delayed-start tofersen patients who originally received placebo. Additionally, the Applicant plans to compare overall survival and function in patients receiving tofersen to available real world evidence of patients living with SOD1-ALS, including natural history data, disease registries, and expanded access patients. However, the comparison of open-label data to an external control may be limited in interpretability and in its ability to serve as confirmatory evidence of a clinical benefit.

Our regulations allow for regulatory flexibility to expedite the development, evaluation, and marketing of new therapies intended to treat persons with life-threatening and severely debilitating illnesses, especially where no satisfactory alternative therapy exists. The 2019 FDA draft guidance, "Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products", states that, "in certain settings, a somewhat greater risk (compared to placebo-controlled or other randomized superiority trials) of false positive conclusions – and therefore less certainty about effectiveness – may be acceptable, when balanced against the risk of rejecting or delaying the marketing of an effective therapy, (…) for an unmet medical need." The guidance provides examples of the use of regulatory flexibility in consideration of alternate trial designs to the standard randomized, double-blind, placebo-controlled study and the use of surrogate or intermediate clinical endpoints under the accelerated approval pathway. Regarding the number of trials considered sufficient to establish effectiveness, the guidance states that a single trial may be sufficient "when a large multicenter trial has demonstrated a clinically meaningful and statistically very persuasive effect on mortality, irreversible morbidity, or prevention of a disease with potentially serious outcome, a second trial would be impracticable or unethical." For rare diseases the guidance notes that, "a second trial may be infeasible in certain rare disease settings where the limited patient populations preclude the conduct of a second trial. In these cases, the substantial evidence of effectiveness would typically be provided by a single trial plus confirmatory evidence."

Given the exceedingly low prevalence of SOD1-ALS, the seriousness of the disease, and the substantial unmet need, we would also like input from the advisory committee members on whether the combination of the existing clinical data from the Phase 3 study and the currently available data from the extension study, accompanied by the reductions of SOD1 and NfL, provide convincing evidence of the effectiveness of tofersen in the treatment of patients with SOD1-ALS.

1.4 Draft Points for Consideration

The Advisory Committee is being asked to consider the following items.

- Whether the available evidence supports that a reduction in NfL observed in tofersen-treated patients with amyotrophic lateral sclerosis (ALS) secondary to a mutation in SOD1 (SOD1-ALS) is reasonably likely to predict clinical benefit for these patients.
- Whether the clinical data from the placebo-controlled study and available long-term extension study results, with additional supporting results from the effects on relevant biomarkers (i.e., changes in NfL and/or reductions in SOD1), provide convincing evidence of the effectiveness of tofersen in the treatment of patients with SOD1-ALS.

- The overall benefit-risk assessment for tofersen in patients with amyotrophic lateral sclerosis (ALS) secondary to a mutation in SOD1 (SOD1-ALS). If the benefit-risk assessment does not appear favorable, consider what additional data would be needed for the risk-benefit assessment to be favorable.
- In these considerations, the AC committee may keep in mind the seriousness of ALS, the rarity of SOD1-ALS and the feasibility of conducting another adequate and well-controlled study in this population, and the unmet need for this population.

2 Introduction and Background

2.1 Background of the Condition/Standard of Clinical Care

ALS is a rapidly progressive and fatal motor neuron disease. It is characterized by the gradual degeneration and death of the motor neurons responsible for voluntary control of muscles. Most cases of ALS are sporadic. Five to ten percent of ALS cases are familial and are associated with approximately 30 different genes. The SOD1 gene is associated with 20% of familial cases and approximately 1-3% of sporadic ALS cases. The mechanism by which mutations in the SOD1 gene cause ALS are not fully understood; the human SOD1 protein is ubiquitously expressed throughout the body and involved in removal of superoxide radicals. In ALS patients with pathogenic SOD1 gene mutations, the toxic accumulation of mutated or misfolded SOD1 protein is the most widely studied mechanistic link of SOD1 mutations and ALS. There are over 200 causative SOD1 mutations associated with ALS that have been identified to date.

ALS patients become progressively weaker, losing the ability to move their bodies. Respiratory muscles are also affected, leading to respiratory failure and the death of most patients within 3 to 5 years from the onset of symptoms. Approximately 10 percent of ALS patients may survive for 10 or more years. Shorter survival is associated with older age at onset, bulbar onset, and faster rate of respiratory dysfunction. One SOD1 mutation, the p.Gly24Asp (G42D, G41D) mutation has a mean disease duration of 23.5 years, whereas the A5V variant SOD1 mutation (also known as p.Ala5Val, ala4val, or A4V), present in approximately 50% of all North American families with identifiable SOD1 variants, is associated with a rapidly progressive disease course, with median survival of 1.2 years and mean disease duration of 1.4 years. Across all reported literature, no A5V carriers have survived more than 4 years.

The incidence of ALS is 2 per 100,000 per year with approximately 6000 new cases per year in the U.S. The estimated prevalence in the U.S. is 5 per 100,000 population with approximately 16,000 cases. Approximately 2% of the ALS population is affected by SOD-1 ALS. The prevalence of SOD1-ALS in the US is estimated at fewer than 500 cases. ALS most frequently affects people between 40 and 70 years of age (median age 55). Familial ALS generally has a 10-year earlier onset than sporadic ALS.

There is no cure for ALS. Most available treatments are intended to relieve symptoms, such as cramps and spasticity, and improve the quality of life. There are three FDA-approved treatments for ALS: riluzole, which was shown to prolong survival by about 3 months and extend the time before ventilatory support is needed; edaravone, which demonstrated a 33% smaller functional decline compared to placebo after 24 weeks in patients within 2 years of diagnosis and with a forced vital capacity (FVC) of at

least 80%; and sodium phenylbutyrate/taurursodiol, which had less worsening in the ALSFRS-R total score from baseline to Week 24 in treated patients compared to placebo-treated patients. Although these therapies may provide some benefit for patients, there is continued need for new treatments for patients living with ALS despite these available therapies. There are no available therapies specifically for patients with SOD1-ALS.

2.2 Background on Neurofilament Light Chain (NfL)

Neurofilament Light Chain (NfL) is one of the neurofilament proteins that are highly expressed in myelinated axons. Elevated levels of NfL in the cerebrospinal fluid (CSF) and blood are found in a variety of neurological disorders including ALS[4] and are a consequence of axonal damage.[5,6,7] Neurofilament levels in the plasma and the CSF, including neurofilament heavy chain (pNF-H) and NfL are significantly elevated in patients with ALS compared to other neurodegenerative diseases.[7,8,9,10] For SOD1-mutation carriers of ALS patients, elevated serum NfL levels have been observed as early as 1 year before symptom onset.[11]

Several independent studies have recently reported that NfL levels are correlated with disease severity, disease progression rate, and survival in patients with ALS.[12,13] A meta-analysis of published literature findings on NfL in ALS demonstrated a correlation between the rate of disease progression and plasma NfL level (Section 3.1.2.2.2.1). Additionally, higher levels of neurofilament were associated with a higher risk of unfavorable clinical outcomes, including death, tracheostomy, and/or permanent ventilation. NfL was reported to have a stronger association than other candidate biomarkers with ALS progression rate and survival.[14] These findings offer support for the utility of NfL as a prognostic biomarker for ALS disease progression and survival. It should also be noted that a reduction of neurofilament levels has been previously reported for approved products for the treatment of other neurological diseases,

[4] Verber NS, Shepheard SR, Sassani M, et al. Biomarkers in Motor Neuron Disease: A State of the Art Review. Front Neurol. 2019;10:291. Published 2019 Apr 3. doi:10.3389/fneur.2019.00291

[5] Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019;90(8):870-881. doi:10.1136/jnnp-2018-320106

[6] Yuan A, Nixon RA. Neurofilament Proteins as Biomarkers to Monitor Neurological Diseases and the Efficacy of Therapies. Front Neurosci. 2021;15:689938. Published 2021 Sep 27. doi:10.3389/fnins.2021.689938

[7] Olsson B, Portelius E, Cullen NC, et al. Association of Cerebrospinal Fluid Neurofilament Light Protein Levels With Cognition in Patients With Dementia, Motor Neuron Disease, and Movement Disorders. JAMA Neurol. 2019;76(3):318-325. doi:10.1001/jamaneurol.2018.3746

[8] Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS One. 2013;8(9):e75091. Published 2013 Sep 20. doi:10.1371/journal.pone.0075091

[9] Behzadi A, Pujol-Calderón F, Tjust AE, et al. Neurofilaments can differentiate ALS subgroups and ALS from common diagnostic mimics. Sci Rep. 2021;11(1):22128. Published 2021 Nov 11. doi:10.1038/s41598-021-01499-6

[10] Heckler I, Venkataraman I. Phosphorylated neurofilament heavy chain: a potential diagnostic biomarker in amyotrophic lateral sclerosis. J Neurophysiol. 2022;127(3):737-745. doi:10.1152/jn.00398.2021

[11] Benatar M, Wuu J, Andersen PM, Lombardi V, Malaspina A. Neurofilament light: A candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. Ann Neurol. 2018;84(1):130-139. doi:10.1002/ana.25276

[12] Lu, Ching-Hua et al. "Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis." Neurology vol. 84,22 (2015): 2247-57. doi:10.1212/WNL.0000000000001642

[13] Dreger M, Steinbach R, Otto M, Turner MR, Grosskreutz J. Cerebrospinal fluid biomarkers of disease activity and progression in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2022;93(4):422-435. doi:10.1136/jnnp-2021-327503

[14] 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. Published 2022 Feb 9. doi:10.1093/braincomms/fcac029

including multiple sclerosis, spinal muscular atrophy, and hereditary transthyretin-mediated amyloidosis,[15,16,17] which provides additional context regarding the use of NfL as a pharmacodynamic biomarker that may correlate with clinical benefit.

2.3 Pertinent Drug Development and Regulatory History

- Tofersen is administered intrathecally (IT) via lumbar puncture at a dose of 100 mg/15 mL. Treatment is proposed with a loading dose of 100 mg administered 14 days apart for 3 doses, followed by a maintenance dose of 100 mg administered every 28 days.
- The proposed indication is the treatment of amyotrophic lateral sclerosis (ALS) in adults with a confirmed mutation of the superoxide dismutase 1 (SOD1) gene.
- The initial investigational new drug application (IND) was opened in 2015 with a first-in-human study in patients with ALS (Study 233AS101 Parts A and B). The Applicant received Fast Track designation at the same time.
- A Type C meeting in 2017 was held to discuss potential endpoints of a Phase 3 study (Study 233AS101 Part C). FDA advised that the preferred primary analysis should include a combined assessment of function and survival, such as the joint-rank analysis, especially given the high likelihood of mortality and missing data in ALS studies.
- The protocol for the pivotal study (Study 101C) was submitted in January 2019. A Type C meeting was also held in May 2019 to discuss the proposed approach to enrich the primary analysis population in Study 233AS101 Part C (Study 101C) based on SOD1 mutation type and prerandomization ALSFRS-R Slope.
- Breakthrough Therapy Designation was denied in April 2020 as the preliminary available data from the Phase 1 study did not demonstrate substantial improvement over existing therapies on one or more clinically significant endpoints.
- A Type C Meeting was held in August 2020 to discuss the proposed design of a Phase 3 Study (Study 233AS303) in presymptomatic carriers of confirmed SOD1 mutations, including discussion of using a prespecified NfL threshold for randomizing presymptomatic patients to drug or placebo prior to development of symptom onset.
- A Type C Meeting was held in September 2021 to discuss the topline results of Study 101C. Although the primary endpoint did not meet statistical significance, the Division agreed at the time that the results suggested a treatment effect, especially in terms of the target engagement (SOD1 reduction) and NfL reduction, and supported plans for continued development.

[15] Darras BT, Crawford TO, Finkel RS, et al. Neurofilament as a potential biomarker for spinal muscular atrophy. Ann Clin Transl Neurol. 2019;6(5):932-944. Published 2019 Apr 17. doi:10.1002/acn3.779

[16] Sormani MP, Haering DA, Kropshofer H, et al. Blood neurofilament light as a potential endpoint in Phase 2 studies in MS. Ann Clin Transl Neurol. 2019;6(6):1081-1089. Published 2019 May 28. doi:10.1002/acn3.795

[17] Ticau S, Sridharan GV, Tsour S, et al. Neurofilament Light Chain as a Biomarker of Hereditary Transthyretin-Mediated Amyloidosis. Neurology. 2021;96(3):e412-e422. doi:10.1212/WNL.0000000000011090

- A Type C Meeting was held in December 2021 regarding possibility of a New Drug Application submission based on the Study 101C data, and the Division indicated that although the data and exploratory analyses appear promising, it would be challenging to support an NDA based on full approval off of the single failed trial.
- A Type B pre-NDA meeting was held in April 2021 to discuss a New Drug Application to support accelerated approval based on change from baseline to Week 28 in plasma NfL.
- NDA was submitted on May 25, 2022, requesting accelerated approval under 21 CFR 314.500 Subpart H, and was granted priority review.
- Additional clinical and clinical pharmacology information was submitted to the NDA on October 5, 2022, and October 7, 2022 which constituted a major amendment to the application, and the review was extended by three months.

3 Summary of Issues for the AC

3.1 Efficacy Issues

The Applicant conducted a single, randomized, double-blind, placebo-controlled, Phase 3 study of tofersen in 108 patients with SOD1-ALS. This study is large given the very low prevalence of SOD1-ALS. The study design was typical of many Phase 3 ALS trials, with a 28-week duration and an acceptable primary endpoint, the ALS Functional Rating Scale-Revised (ALSFRS-R). Randomization was stratified by "fast progressor" and "non-fast progressor". Fast progressors were defined by genetic mutation and pre-randomization slope on the ALSFRS-R, and this fast progressor group formed the primary analysis population, also referred to as the modified intention-to-treat (mITT) population. The selection of enrichment factors was based on available scientific knowledge at the time the study was designed and pre-randomization slope on the ALSFRS-R is commonly used as an enrichment criteria in ALS trials.

This study failed to show a statistically significant difference between the tofersen and placebo groups for the primary or secondary endpoints in the prespecified primary analysis. Although the results numerically favored tofersen for all endpoints, the estimated treatment effect on the primary endpoint ALSFRS-R at week 28 in the primary mITT population was 1.2, 95% CI: -3.2, 5.5, with p=0.97 using the primary joint rank analysis or p=0.60 using a supportive ANCOVA analysis. The biomarker data from the study indicate target engagement of the therapy with reduction of the SOD1 protein in CSF, and a reduction in NfL, a biomarker of neurodegeneration that has been shown to be correlated with disease progression and prognosis in patients with ALS.

The Applicant has asserted that there are potential reasons that the study may have failed to detect a treatment effect, if there is one, including: a slower than predicted rate of decline in the study population, enrichment criteria based on genetic mutation and pre-randomization ALSFRS-R slope that did not adequately identify a fast progressing population, baseline imbalances in NfL, and a study duration that was likely too short to detect a treatment effect given the proposed mechanism of action. Many of these issues are discussed further in the sections that follow. Additionally, Agency review found that the decline in both the placebo and treatment groups was much less than expected, leading to the study being greatly underpowered.

The sponsor has proposed the plasma NfL may be a reasonably likely surrogate endpoint to support AA for tofersen in the treatment of SOD-1ALS, based on the following aspects:

- There is mechanistic evidence that tofersen reduces SOD1 protein, the intended target of the drug and known contributor to the pathophysiology of neuronal degeneration in patients with SOD1-ALS, and also reduces NfL, a biomarker of neurodegeneration that is known to be substantially elevated in patients with ALS and predictive of disease progression.
- Evidence from the literature and tofersen clinical program have demonstrated the prognostic value of plasma NfL in predicting disease progression and survival in ALS.
- An observed correlation between reduction in NfL and a slowing of decline on clinical outcomes such as the ALSFRS-R, and a causal inference model, despite statistical limitations.

An open-label extension study provides additional data on treatment with tofersen over a longer period of time. In the extension study, patients remained blinded to their original treatment assignment in the Phase 3 study. The Applicant has presented post hoc, exploratory analyses (including NfL as a covariate in the statistical model, even as not prespecified) that suggest a possible clinical benefit with a longer duration of treatment, and analyses that purport to show that a reduction in NfL may be predictive of clinical benefit. Based on the observed data of ALSFRS-R total score, the early-start tofersen group has shown numerically less decline in ALSFRS-R total score as compared to delayed-start group, which is consistent from Week 28 to Week 52. However, these OLE analyses have limitations detailed below that make interpretation of the results challenging.

The efficacy issues for consideration are:

- Whether the available evidence supports that a reduction in NfL observed in tofersen-treated patients with amyotrophic lateral sclerosis (ALS) secondary to a mutation in SOD1 (SOD1-ALS) is reasonably likely to predict clinical benefit for these patients.
- Whether the clinical data from the placebo-controlled study and available long-term extension study results, with additional supporting results from the effects on relevant biomarkers (i.e., changes in NfL and/or reductions in SOD1), provide convincing evidence of the effectiveness of tofersen in the treatment of patients with SOD1-ALS.

3.1.1 Clinical Efficacy Assessment

3.1.1.1 Sources of Data for Efficacy

Study Design

Study 233AS101 Part C (referred to as Study 101C) was a multicenter (32 study sites), multinational (9 countries), randomized, double-blind, placebo-controlled, Phase 3 study of tofersen in adult subjects with ALS and a confirmed pathogenic or likely pathogenic SOD1 mutation. In addition to Study 101C, data from the open-label extension study, Study 102 (See Table 1), is also proposed to contribute to the evidence for effectiveness.

The primary objective of Study 101C was to evaluate the efficacy of tofersen and the secondary objective was to evaluate the safety, tolerability, pharmacodynamics (PD), and biomarker effects of tofersen administered to adult participants with SOD1-ALS. Study 101C randomized 108 adult subjects (72 tofersen and 36 placebo) with SOD1-ALS to receive treatment with tofersen or placebo for 24 weeks. The total study duration was up to approximately 32 weeks: including up to a 4-week screening period, a 24-week treatment period (consisting of 3 loading doses of tofersen administered approximately once every 2 weeks, followed by 5 maintenance doses of tofersen, administered approximately once every 4 weeks), and a follow-up visit 4 weeks after the last dose.

Study 101C evaluated clinical function through the ALS Functional Rating Scale-Revised (ALSFRS-R) total score, respiratory function through slow vital capacity (SVC), quantitative strength measurement

through handheld dynamometry (HHD), and time to death or permanent ventilation. Additional assessments included biomarkers such as blood and cerebrospinal fluid (CSF) concentrations of neurofilament light chain (NfL) and misfolded or mutant SOD1 protein.

In Study 101C, patients were randomized 2:1 (active: placebo) and stratified according to the following factors: whether a subject meets the prognostic enrichment criteria for rapid disease progression and edaravone or riluzole use (with three categories: edaravone use with or without riluzole, riluzole use only, and neither edaravone nor riluzole use). There were two subgroups: subjects enriched for rapid disease progression based on pre-randomization ALSFRS-R slope and mutation, and all others. The subgroup of subjects enriched for rapid disease progression made up the mITT population (N = 60) that was used for the primary efficacy analysis.

The mean (SD) duration in the study (starting from the first dose until the end of the study) was 190 (29) days in the tofersen group and 195 (16) days in the placebo group.

Patients who completed Study 101C were enrolled in Study 102, an ongoing open-label extension study. Patients, site staff, and all vendors remained blinded to the original individual treatment assignments in Study 101C. Study 102 is ongoing, and planned to continue through mid-2024, at which point participants will have been followed for approximately 3-7 years, depending on time of enrollment. The interim data cuts of Study 102 were performed on July 16, 2021, at the time of completion of Study 101C, and January 16, 2022, when all participants from Study 101C had at least 12 months of follow-up (Week 52 analyses). An additional safety-only data cut was conducted on July 15, 2022, when all participants had the opportunity for 18 months of follow-up. This data provided the basis for the 120-day safety update.

Table 1 Clinical Studies/Trials Submitted in Support of Efficacy and/or Safety Determinations for Tofersen

Study/Trial Identifier	**Study/Trial Population**	**Study/Trial Design**	**Regimen (Number Treated), Duration**	**Primary and Key Secondary Endpoints**	**Number of Subjects Planned; Actual Randomized**	**Number of Centers and Countries**
233AS101 Part C	Adults with ALS and SOD1 mutation	randomized, double-blind, placebo-controlled	Drug: tofersen Dosage: 100 mg intrathecal Number treated: 108 Duration: 28 weeks	Primary: change from baseline in ALSFRS-R Secondary: Change from baseline to Week 28 in percent predicted SVC, total CSF SOD1 protein, plasma NfL and HHD megascore	108 participants randomized and all randomized participants dosed	32 study sites in 9 countries

233AS102	Adults with ALS and SOD1 mutation	open-label extension	Drug: tofersen Dosage: 100 mg intrathecal Number treated: 139 Duration: up to 360 weeks	Primary: ALSFRS-R Secondary: SVC, HHD, time to death, CSF SOD1, CSF and plasma NfL	139 subjects enrolled, received at least 1 dose of study treatment, and were included in the safety population	37 study sites in 13 countries

Pre-Specified Statistical Methods

The final version of the statistical analysis plan (SAP) prior to database lock for Study 101C (which occurred on August 16, 2021) was SAP V2, which was finalized on August 14, 2021. This section describes the analysis methods pre-specified in SAP V2 for select endpoints.

The primary analysis of change from baseline in ALSFRS-R at Week 28 was based on an ANCOVA of joint ranked scores[18] in the mITT population, adjusting for baseline ALSFRS-R, edaravone or riluzole use, and time since symptom onset. Multiple imputation (MI) was used for missing data in survivors. The analysis of the secondary endpoint, percent predicted SVC at Week 28, was based on an ANCOVA of joint ranked scores in the mITT population, adjusting for baseline percent predicted SVC, baseline ALSFRS-R, edaravone or riluzole use, and time since symptom onset, with MI for missing data. The analysis of the secondary endpoints time to death or permanent ventilation and time to death were based on a log-rank test stratified by riluzole or edaravone use, and a Cox proportional hazards model adjusting for time since symptom onset, baseline ALSFRS-R, and edaravone or riluzole use was used to estimate hazard ratios and CIs.

The mITT population was used for all analyses of primary and secondary endpoints. The SAP stated that descriptive analyses may be conducted for the non-mITT and ITT populations but that there would be no formal hypothesis testing.

A sequential testing strategy was used to control the Type I error probability across the multiple endpoints, with testing of secondary endpoints in the following order (if the primary analysis was statistically significant): change from baseline (i.e., ratio) to Week 28 (Day 197) in total CSF SOD1 protein; change from baseline (i.e., ratio) to Week 28 (Day 197) in NfL in plasma; change from baseline to Week 28 (Day 197) in SVC; change from baseline to Week 28 (Day 197) in HHD megascore to assess muscle strength, as measured by the HHD device; time to death or permanent ventilation, defined as

[18] The joint rank methodology (Berry 2013) allows for a statistical test of the treatment effect on the endpoint while accounting for truncation of data due to deaths. In this analysis, a subject's joint rank score is calculated by comparing each subject to every other subject in the study, resulting in a score of +1 if the outcome was better than the subject being compared, -1 if worse, and 0 if the same. The subject's score is then be calculated by summing their comparison to all the other subjects in the study.

the time to the earliest occurrence of death or permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days); and time to death.

The Study 102 SAP V2 (this was the final version of the OLE SAP prior to any unblinding; finalized on August 12, 2021) and the integrated summary of efficacy (ISE) SAP V2 (this was the final version of the ISE SAP prior to any unblinding; finalized on August 14, 2021) also specified endpoints, objectives, and analyses for Study 102 (the OLE), and for combined data from Study 101C and Study 102. The primary objective of Study 102 was to evaluate long-term safety and tolerability of tofersen, with adverse events and serious adverse events specified as primary endpoints. Secondary endpoints included PK endpoints, biomarker and PD endpoints, and efficacy endpoints. Efficacy endpoints included changes over time in ALSFRS-R, SVC, and HHD, time to death or permanent ventilation, and time to death. For ALSFRS-R, descriptive statistics were to be calculated at different time points, along with an ANCOVA, with MI for missing data. For time to death or permanent ventilation and time to death, Kaplan-Meier estimates were to be calculated, along with a Cox proportional hazards model. The pre-specified adjustment covariates for the ANCOVA and Cox models were the same as in the double-blind phase. Given the design and objectives of Study 102 and that efficacy analyses of the OLE data were not part of the planned multiple testing strategy for Study 101C, these efficacy analyses are exploratory in nature.

Additional Applicant Analyses

The Applicant has also conducted a variety of additional analyses of data from the double-blind phase and the OLE phase based on statistical methods that were determined after access to unblinded data. The Applicant finalized an additional SAP (V3) on February 2, 2022. This was after the endpoints of time to death, time to death or permanent ventilation, and ALSFRS-R change through Week 40 in the combined Study 101 Part C and OLE dataset had already been unblinded, analyzed, and presented in the November 2021 Type C meeting briefing package. These additional analyses carried out by the Applicant included changes to pre-specified analysis methods such as a focus on the ITT rather than mITT population, replacing time since symptom onset with baseline NfL as an adjustment covariate (or use of < vs. ≥ median baseline NfL as a stratification factor for log-rank tests), and changes to the MI model (adding NfL as a covariate). Baseline NfL was not included as an adjustment covariate or stratification factor in primary and secondary endpoint analyses in either SAP V2 or the earlier V1 (dated 2 months after the trial started).

3.1.1.2 Clinical Efficacy Outcomes

3.1.1.2.1 Results of Study 101C and Study 102 Open-label extension

Study 101C Participant Disposition and Baseline Disease Characteristics

Table 2 compares baseline disease characteristics between Study 101C participants in the tofersen and placebo populations. The two groups are generally well balanced; however, the baseline plasma NfL levels are noted to be higher in the tofersen arm than the placebo arm in the mITT population. There are some high placebo values which affect the mean statistic for baseline NfL. The mean is often not used,

nor the best summary statistic for a highly skewed distribution such as the baseline NfL, as noted in the analysis plan. The scale for analysis of NfL according to the analysis plan is the log scale. The baseline NfL means on the log scale are 4.52 (S.D.=0.92) for placebo and 4.82 (S.D.=0.67) for tofersen in the mITT population. There are also slight imbalances in the group proportions with the p.Ile114Thr gene in all populations (28% placebo vs. 14% Tofersen in ITT), more upper limb onset in the placebo group in the mITT population (67% vs. 49%) and a numerically greater pre-randomization decline slope for placebo (1.51 vs. 1.34) in the mITT population.

The overall rate of missing data was relatively low in the double-blind (DB) study, 15% (6/39) in the tofersen group and 10% (2/21) in the placebo group.

Table 2 Study 101 Part C Baseline Disease Characteristics

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

[1] Most common mutations, i.e., n > 4

Source: Applicant's Clinical Study Report, page 67

Study 101C Results (Based on Pre-specified Statistical Analyses)

This section describes results for the primary and secondary endpoints in Study 101C based on the pre-specified statistical methods from SAP V2. Results are shown for the primary mITT population, as well as the ITT population (which was specified as exploratory).

The primary analysis of Study 101C did not succeed in the primary mITT population (i.e., fast progressors; N = 60). The mean change in ALSFRS-R at Week 28 on tofersen was -8.1 as compared to -7.0 on placebo, for an estimated treatment difference of 1.2 (95% CI: -3.2, 5.5), with the primary joint rank test p=0.97 and supportive ANCOVA p=0.60.

Table 3 Mean Change from Baseline in ALSFRS-R at Week 28 in Study 101 Part C Based on Pre-Specified Analysis Methods

Population	Summary Measure	Placebo N=21 mITT N=15 non-mITT	Tofersen N=39 mITT N=33 non-mITT	Week 28 Mean Difference (95% CI) ANCOVA+MI
mITT (Primary Analysis)	Baseline Mean Week 28 LS Mean Change	35.4 -8.1	36.0 -7.0	1.2 (-3.2, 5.5) Joint Rank p=0.97 ANCOVA+MI p=0.60
Non-mITT	Baseline Mean Week 28 LS Mean Change	39.9 -2.7	38.1 -1.3	1.4 (-1.1, 3.9) Joint Rank p=0.998 ANCOVA+MI p=0.27
ITT	Baseline Mean Week 28 LS Mean Change	37.3 -5.8	36.9 -4.5	1.4 (-1.3, 4.1) Joint Rank p=0.91 ANCOVA+MI p=0.32

Source: Statistical Reviewer's Analysis

Abbreviations: LS=least squares; CI=confidence interval; MI=multiple imputation

Based on the prespecified testing hierarchy, after the primary endpoint did not succeed, statistical testing stopped.

Change from baseline to Week 28 in percent predicted SVC showed nominally less decline in the tofersen group (adjusted mean -14.3) compared to the placebo group (adjusted mean -22.2) in the mITT population, but this was not statistically significant (7.9% treatment difference, nominal p = 0.32). Change from baseline to Week 28 in the HHD megascore also did not show a statistically significant difference for the tofersen group (adjusted mean -0.34) compared to the placebo group (adjusted mean -0.37) in the mITT population, with a treatment difference of 0.02 (nominal p = 0.84).

In the exploratory ITT population, the comparison of change from baseline to Week 28 in ALSFRS-R between the tofersen group (adjusted mean -4.5) and the placebo group (adjusted mean -5.8) showed a treatment difference of 1.4 (95% CI: -1.3, 4.1), p=0.32 (p=0.91 for joint rank analysis). The comparison of

change from baseline to Week 28 in percent predicted SVC between the tofersen group (adjusted mean -7.9) and the placebo group (adjusted mean -14.8) showed a treatment difference of 6.9 (95% CI: -0.1, 13.8), p=0.05 (p=0.15 for joint rank analysis). The comparison of change from baseline to Week 28 in the HHD megascore between the tofersen group (adjusted mean -0.23) and the placebo group (adjusted mean=-0.29) showed a treatment difference of 0.06 (95% CI: -0.09, 0.21), p=0.44.

Time to death or permanent ventilation, and time to death were not assessed due to lack of events. There was only 1 death during the double-blind period.

Other secondary endpoints were the CFB (change from baseline) at Week 28 in total SOD1 (superoxide dismutase 1) concentration in CSF (cerebrospinal fluid) and NfL (neurofilament light chain) concentration in plasma. A reduction in total CSF SOD 1 protein was observed at Week 28 in the tofersen group compared to the placebo group (38% difference in geometric means ratio for tofersen to placebo, nominal $p < 0.0001$) in the mITT population. A reduction in plasma NfL was observed at Week 28 in the tofersen treatment arm compared to placebo (67% difference in geometric mean rations for tofersen to placebo, nominal $p < 0.0001$) in the mITT population. The significance of these changes in CSF and plasma biomarkers are discussed further in Section 3.1.2.1.

Study 102 Disposition

Participant disposition in the OLE period (Study 102) for Study 101C participants is shown in Table 4. The table shows that for the ITT population, 88-89% of the placebo and tofersen groups participated in study 102. For the mITT population, 19/21 [90%] placebo and 33/39 [85%] tofersen subjects participated in Study 102.

Table 4 Participant Disposition of Integrated Analysis of Studies 101 Part C and 102

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

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

Source: Applicant's Summary of Clinical Efficacy, pg 74.

Study 102 Results (Based on Pre-specified Statistical Analyses)

As noted in the statistical methods section, efficacy analyses in Study 102 were exploratory in nature, and analyses were planned at multiple time points during the OLE. This section focuses on results of pre-specified analyses of ALSFRS-R, percent predicted SVC, and NfL through Week 52, as well as analyses of time to death or permanent ventilation and time to death through the final data cutoff in January 2022.

At Week 52 of the OLE, results for ALSFRS-R were similar to those observed at Week 28 of the double-blind period, with roughly similar estimates and lack of statistical evidence of differences between arms. In the mITT population, the estimated mean difference was 2.5 (95: CI: -3.2, 8.3), p=0.39 based on the planned ANCOVA + MI approach. In the ITT population, the estimated difference was 2.7 (95% CI: -0.9, 6.2), p=0.14. The ANCOVA analysis of joint rank scores was not pre-specified for the OLE but it yielded similar results, with a difference of 1.1 (95: CI: -8.7, 10.8), p=0.83 in the mITT population, and 4.2 (95% CI: -7.4, 15.8), p=0.48 in the ITT population. Only 28/36 (78%) placebo/delayed-start tofersen and 57/72 (79%) early tofersen subjects had non-missing ALSFRS-R scores at Week 52 in the ITT population for the final January 2022 data cut (note that 1 [2.8%] placebo and 4 [5.6%] tofersen subjects had died by Week 52).

Table 5 Mean Change in ALSFRS-R at Week 52 From Study 101 Part C Baseline Through Studies 101 Part C and 102 Based on Pre-Specified Analysis Methods

Population	Summary Measure	Placebo N=21 mITT N=15 non-mITT	Tofersen N=39 mITT N=33 non-mITT	Week 52 Mean Difference (95% CI) ANCOVA+MI
mITT	Baseline Mean Week 52 LS Mean Change	35.4 -12.3	36.0 -9.7	2.5 (-3.2, 8.3) Joint Rank p=0.83 ANCOVA+MI p=0.39
Non-mITT	Baseline Mean Week 52 LS Mean Change	39.9 -3.9	38.1 -1.2	2.6 (-0.7, 6.0) Joint Rank p=0.90 ANCOVA+MI p=0.12
ITT	Baseline Mean Week 52 LS Mean Change	37.3 -9.3	36.9 -6.6	2.7 (-0.9, 6.2) Joint Rank p=0.48 ANCOVA+MI p=0.14

Source: Statistical Reviewer's Analysis

Abbreviations: LS=least squares; CI=confidence interval; MI=multiple imputation

There were slightly more favorable results for the first key secondary endpoint, percent predicted SVC during the OLE than the double-blind period, although differences were not nominally statistically significant. In the mITT population, the estimated mean difference was 8.7 (95% CI: -5.7, 23.2), p=0.23 based on the planned ANCOVA + MI approach. In the ITT population, the estimated difference was 7.5 (95% CI: -0.7, 15.7), p=0.07. The ANCOVA analysis of joint rank scores was not pre-specified for the OLE but it yielded similar results, with a difference of 3.9 (95% CI: -6.1, 14.0), p=0.44 in the mITT population, and 8.9 (95% CI: -3.6, 21.5), p=0.16 in the ITT population.

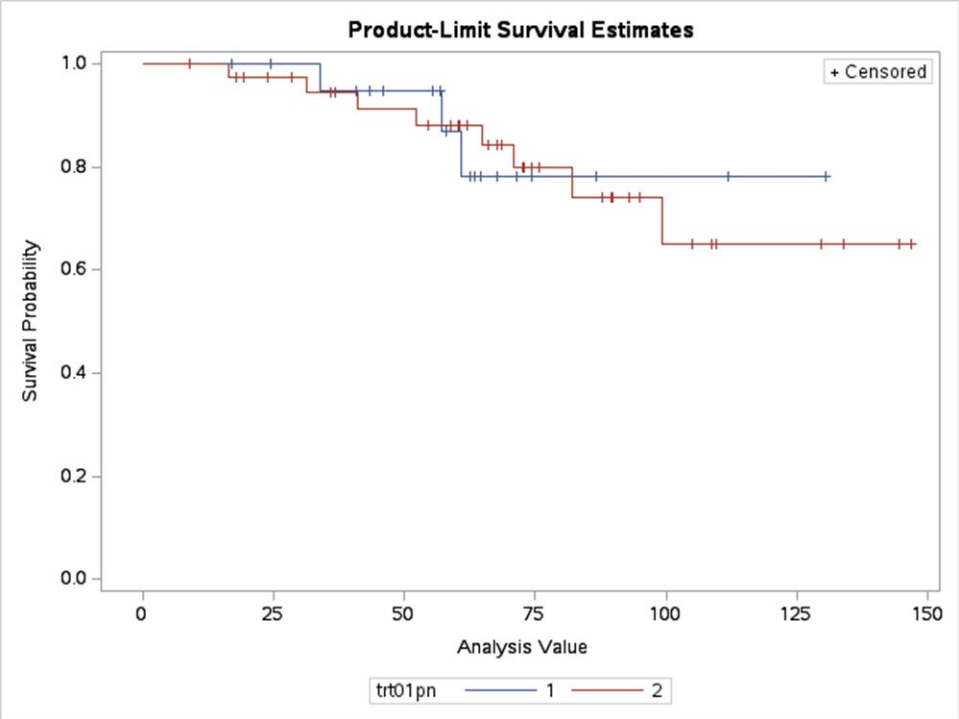
Results for the secondary endpoints CSF and NfL continued to show reduction with tofersen, with participants in the tofersen group maintaining their lowered levels, and participants in the placebo/delayed-start tofersen group showing reduced levels. Results are discussed in more detail in Section 3.1.2.1.

For time to death or permanent ventilation and time to death through all follow-up (up to 150 weeks), trends went in different directions in the mITT and ITT populations. Uncertainty around estimates was large due to the small numbers of events, and differences between arms were not nominally statistically significant. In the mITT population, 12/39 (30.8%) subjects on tofersen and 5/21 (23.8%) subjects on placebo/delayed-start tofersen died or went on permanent ventilation, for an estimated hazard ratio (HR) of 1.69 (95% CI: 0.53, 5.40), p=0.38. For time to death alone, 8/39 (20.5%) subjects on tofersen and 3/21 (14.3%) subjects on placebo/delayed-start tofersen died, for an estimated HR of 1.67 (95% CI: 0.39, 7.10), p=0.49. These numerical trends favored placebo over tofersen in the mITT population. In the ITT population, 12/72 (16.7%) subjects on tofersen and 8/36 (22.2%) subjects on placebo/delayed-start

tofersen died or went on permanent ventilation, for an estimated HR of 0.70 (95% CI: 0.28, 1.75), p=0.45. For time to death alone, there were 8/72 (11.1%) events on tofersen as compared to 6/36 (16.7%) on placebo/delayed-start tofersen, for a HR of 0.64 (95% CI: 0.22, 1.88), p=0.41. Kaplan-Meier plots of the probability of survival over time in the mITT and ITT populations are shown in Figure 1 and Figure 2.

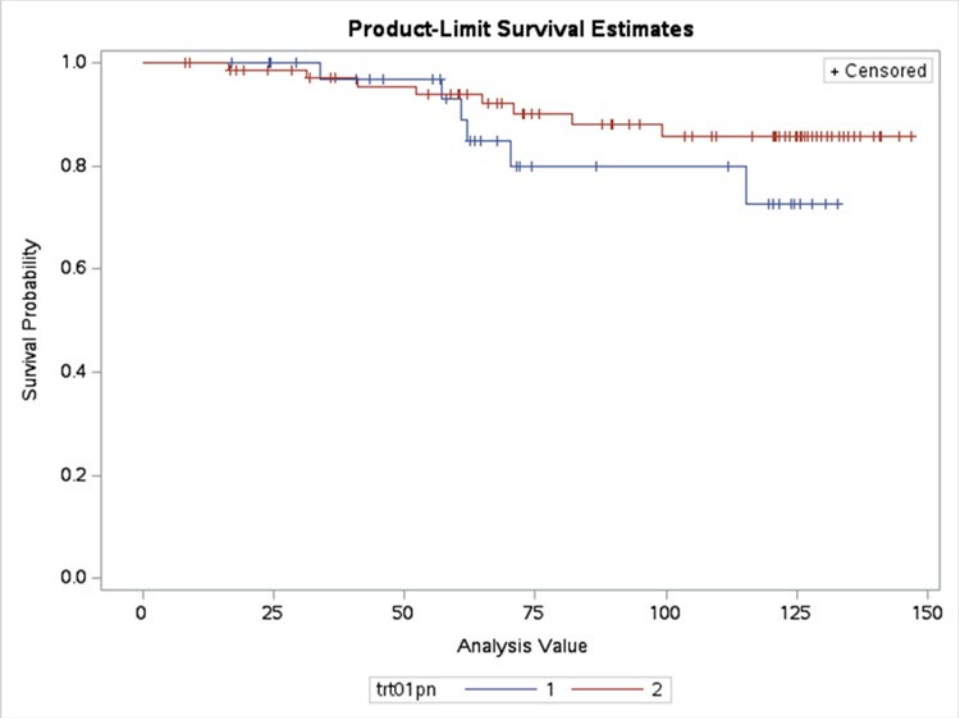
It is difficult to adjust for the multiplicity of survival analyses that have been conducted, as multiple time-to-event endpoints have been analyzed by the Applicant at several calendar times, in both the mITT and ITT populations, and with and without post-withdrawal vital status follow-up. The potential for data-driven choices of analyses to emphasize can induce bias and create challenges in interpreting results. Also note that follow-up is not complete in the analyses using post-withdrawal vital status follow-up. In particular, 5 (7%) of tofersen and 3 (8%) of tofersen placebo/delayed-start tofersen subjects did not have post-withdrawal vital status collected. There is also additional missingness for permanent ventilation in the time to death or permanent ventilation analyses given that events after withdrawal or for non-participants in study 102 could have been missed. With the low number of events, this incomplete follow-up could be impactful.

Figure 1 Kaplan Meier Curves for Time to Death in mITT population (Adjudicated Events)



Source: Statistical Reviewer's Analysis note: trt01pn 1=Placebo/delayed-start Tofersen 2= early Tofersen; Analysis Value is the Time of Event in Weeks

Figure 2 Kaplan Meier Curves for Time to Death in ITT population (Adjudicated Events)



Note: trt01pn 1=Placebo/delayed-start Tofersen 2= early Tofersen

Source: Statistical Reviewer's Analysis

Additional Analyses of Study 101C and the OLE (Study 102) by the Applicant

As noted above in the statistical methods section, the Applicant has conducted a variety of additional analyses based on statistical methods determined after access to unblinded data. These additional analyses carried out by the Applicant included changes to pre-specified analysis methods such as a focus on the ITT rather than mITT population, replacing time since symptom onset with baseline NfL as an adjustment covariate, and changes to the MI model (adding NfL as a covariate).

Results for the primary endpoint in Study 101C, change from baseline in ALSFRS-R at Week 28, using these post hoc analysis methods were more favorable for tofersen than the pre-specified primary analysis but still did not reach nominal statistical significance, with an estimated mean difference of 2.1 (95% CI: -0.33, 4.54), p=0.09.

Results for ALSFRS-R, percent predicted SVC, and HHD Megascore at Week 52 of the OLE are shown in Table 6. Results for survival endpoints through the OLE are shown in Table 7. These analyses show trends in favor of tofersen and some of these analyses achieve nominal statistical significance. However, these results are very challenging to interpret, as post hoc potentially data-driven modeling choices can induce substantial bias toward greater effect sizes than the truth. Of particular note is that pre-specification of covariates is critical for the validity of models with covariate adjustment. The FDA draft guidance for industry *Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products* (2021) states that "Sponsors should prospectively specify the covariates and the mathematical

form of the covariate adjusted estimator in the statistical analysis plan before any unblinding of comparative data. FDA will generally give more weight in review to the prespecified primary analysis than to post-hoc analyses using different models or covariates." It should be noted that these analyses also ignore deaths, and there were four deaths on tofersen vs. one death on placebo/delayed-start tofersen prior to Week 52 (all were in the mITT population).

The Applicant has provided some reasonable scientific justifications for the modeling choices, for example, providing data to support that NfL is prognostic of functional decline and that the pre-specified mITT primary analysis population defined by genetic mutation and pre-randomization ALSFRS-R slope may not have done the best job of identifying fast progressors (3.1.2.2.2.2). However, adjustment for prognostic covariates is not necessary for valid inference on treatment effects—the pre-specified analyses of this trial were statistically valid and should be weighted heavily. Furthermore, there are always a variety of alternative modeling choices that can be justified scientifically after viewing the data from a trial, and it is clear that at least part of the reason why these analyses are being explored is data-driven, i.e., due to the lack of evidence observed in the pre-specified analyses of both the double-blind and OLE periods.

Table 6: Change in ALSFRS-R, SVC, and HHD Megascore From Study 101 Part C Baseline in Studies 101 Part C and 102 (ITT Population) Based on Applicant Analysis Methods Determined after Data Unblinding

4a) ALSFRS-R

		ISE Based on Jul 2021 Data cut*	**ISE Based on Jan 2022 Data cut****
	Endpoint	Early-start tofersen (n=72) vs. placebo → delayed-start tofersen (n=36) Change from baseline to **Week 40**	Early-start tofersen (n=72) vs. placebo → delayed-start tofersen (n=36) Change from baseline to **Week 52**
ITT	**Adjusted for baseline plasma NfL** N: Tof; Placebo Adjusted means: Tof; Placebo Tofersen-placebo: adjusted mean difference (95% CI) p-value (ANCOVA+MI)	 72; 36 -5.8; -8.5 2.6 (-0.4, 5.7) N/A	 72; 36 -6.0, -9.5 3.5 (0.4, 6.7) 0.0272

Source: Summary of Clinical Efficacy, table 19, p. 76.

4b)SVC percent-predicted

		ISE Based on Jul 2021 Data cut*	**ISE Based on Jan 2022 Data cut****
	Endpoint	Early-start tofersen (n=72) vs. placebo → delayed-start tofersen (n=36) Change from baseline to **Week 40**	Early-start tofersen (n=72) vs. placebo → delayed-start tofersen (n=36) Change from baseline to **Week 52**
ITT	**Adjusted for baseline plasma NfL** N: Tof; Placebo Adjusted means: Tof; Placebo Tofersen-placebo: adjusted mean difference (95% CI) p-value (ANCOVA+MI)	 72; 36 -8.6, -20.8 12.2 (4.3, 20.1) N/A	 72; 36 -9.4; -18.6 9.2 (1.7, 16.6) 0.0159

Source: Summary of Clinical Efficacy, table 20, p. 76.

4c) HHD Megascore

		ISE Based on Jul 2021 Data cut*	**ISE Based on Jan 2022 Data cut****
	Endpoint	Early-start tofersen (n=72) vs. placebo → delayed-start tofersen (n=36) Change from baseline to **Week 40**	Early-start tofersen (n=72) vs. placebo → delayed-start tofersen (n=36) Change from baseline to **Week 52**
ITT	**Adjusted for baseline plasma NfL** N: Tof; Placebo Adjusted means: Tof; Placebo Tofersen-placebo: adjusted mean difference (95% CI) p-value (ANCOVA+MI)	 72; 36 -0.21; -0.51 0.30 (0.09, 0.51) N/A	 72; 36 -0.17; -0.45 0.28 (0.047, 0.517) 0.0186

Source: Summary of Clinical Efficacy, table 19, 20, 21, p. 76.

Table 7 Time-to-Event Analyses in Studies 101 Part C and 102 (ITT Population) Based on Applicant Analysis Methods Determined after Data Unblinding

	ISE Based on Jan 2022 Data cut	
Endpoint	**Early-start tofersen 100 mg (n=72)**	**Placebo/delayed-start tofersen 100 mg (n=36)**
Time to death or permanent ventilation		
Number of events/Total number subjects (%)	12/72 (16.7%)	8/36 (22.2%)
Hazard ratio (95% CI)*		0.36 (0.137, 0.941)
Cox regression p-value*		0.0373
Log-rank test p-value**		0.0687
Time to death		
Number of events/Total number subjects (%)	8/72 (11.1%)	6/36 (16.7%)
Hazard ratio (95% CI)*		0.27 (0.084, 0.890)
Cox regression p-value*		0.0313
Log-rank test p-value**		0.0879
Time to death with additional post-withdrawal vital status data****		
Number of events/Total number subjects (%)	12/72 (16.7%)	11/36 (30.6%)
Hazard ratio (95% CI)*		0.24 (0.096, 0.602)
Cox regression p-value*		0.0023
Log-rank test p-value**		0.0096
Time to death, permanent ventilation, or withdrawal due to disease progression		
Number of events/Total number subjects (%)	18/72 (25.0%)	13/36 (36.1%)
Hazard ratio (95% CI)*		0.38 (0.180, 0.821)
Cox regression p-value*		0.0135
Log-rank test p-value**		0.0217

Note: Analysis for Studies 101 Part C & 102 is based on January 2022 data cut and was prespecified. All p-values are nominal.

Time to death or permanent ventilation is defined as the time from first dose to death or permanent ventilation (>= 22 hours of mechanical ventilation [invasive or noninvasive] per day for >= 21 consecutive days), whichever comes first. Participants who do not meet the endpoint definition are censored at the participant's last known alive date. Similarly, time to death, permanent ventilation 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 CRF.

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

**Based on a log rank test stratified by median baseline plasma NfL.

Note: Median time was not estimable.

****Analysis incorporates vital status data obtained after discontinuation from Studies 101 Part C or 102 for participants who discontinued for reasons other than death.

Source: Summary of Clinical Efficacy, table 22, p. 89.

3.1.1.2.2 Statistical Comments on the Results of Study 101C and Study 102 Open-label Extension

This section focuses on statistical issues with the primary and secondary clinical endpoint results of Study 101C and its OLE (Study 102).

The primary analysis of Study 101C did not provide evidence of a treatment effect for tofersen. There was also no evidence of an effect on ALSFRS-R at Week 28 in the (exploratory) ITT population, nor was there evidence of effects on the secondary endpoints of percent SVC or HHD megascore based on the pre-specified analyses of the 28-week double-blind period (in either the mITT or ITT population). Results for ALSFRS-R, SVC, and HHD megascore, as well as results for time to death and time to death or permanent ventilation, were also explored in the OLE. Based on the pre-specified analyses, while there were some trends toward benefit, results did not achieve nominal statistical significance. This includes analyses in the (exploratory) ITT population. In addition, it is challenging to interpret the OLE results given the exploratory nature of this phase of the study and the lack of evidence in the primary analysis of the double-blind phase.

The Applicant has emphasized additional analyses that provide more favorable results for tofersen. However, these analyses were based on methods that deviated from what was pre-specified and were determined after data unblinding. For example, the ALSFRS-R analysis included a shift from the mITT to ITT population, a change in covariates (replacing time since symptom onset with baseline NfL), and changes to the MI approach (adding baseline NfL). The Applicant provides some scientific justification for the modeling changes, and some of these post hoc results seem promising. However, due to the lack of evidence observed in the pre-specified analyses of both the double-blind and OLE periods, and the potential data-driven nature of these additional analyses, the results are very challenging to interpret.

We also note additional issues with design considerations for Study 101C and Study 102 (refer to issues with sample size planning and issues with method of imputation of missing data in the Appendix Section 5.3). One reason the study failed may have been that decline in both the placebo and treatment groups was much less than expected. The sample size justification was based on an assumed mean slope of decline of -3.83 per month for the placebo participants (i.e., 24.7-point decline over 28 weeks) and -0.74 per month for the tofersen 100 mg participants (approximately a 4.8 decline over 28 weeks), with a pooled SD of 3.166. The actual observed decline over 28 weeks was approximately an 8.1 decline in the placebo arm and a 7-point decline in the tofersen arm, a mean difference of about 1 point instead of the difference of 20 that was assumed. The survival rate in both arms combined was 59/60 (one death in the tofersen arm). Assuming these observed rates of decline and a common survival rate of 59/60 and leaving the other assumptions unchanged, a future study would need 12,000 participants to achieve 80% power to detect a mean difference of 1.1 points decline in ALSFRS-R, noting the uncertainty about the effect of a 1.1-point change. Thus, either tofersen has very small effect or the trial is severely underpowered; both can occur.

3.1.1.3 Survival in Patients with A5V Variant SOD1 mutation

The Applicant has also presented data regarding survival in patients with the A5V variant enrolled in the tofersen clinical studies. the A5V variant (also known as p.Ala5Val, ala4val, or A4V) is associated with a rapidly progressive disease course; a recent chart review of genetically confirmed cases of SOD1-ALS in North America confirmed prior reports of median survival of 1.2 years from disease onset in patients with the A5V mutation (n = 63).[19,20] There are published reports of 2 single patients with an A5V mutation who have lived beyond 3 years, with the longest reported survival of 4 years from time of symptom onset.[19,21] The Applicant also notes that, as of September 20, 2022, a single patient has exceeded the longest known survival at 4.3 years, and another two patients ongoing in the study are currently at 2.4 and 3.6 years since symptom onset, respectively. The median disease duration in participants who have received at least 1 dose of tofersen (n = 16) is 1.7 years (range 0.9-4.4 years). The current disease duration for these patients appears notable; however, the data are too limited to draw any conclusions about the role of tofersen.

3.1.2 Biomarker Assessment

3.1.2.1 Biomarker Overview

<u>NfL in Plasma Results</u>

Change in NfL (i.e., ratio to baseline) at Week 28 in the mITT population was the second endpoint listed among the secondary objectives after the primary and four key secondary endpoints included in the multiple testing hierarchy of Study 101 Part C. Plasma NfL were sampled before dosing at each visit in which treatment was administered (Day 1, 15, 29, and every 4 weeks thereafter) and at the final visit (4 weeks after last dose).

The Applicant's pre-specified secondary analysis of NfL in the mITT population yielded an estimated geometric mean ratio: 0.33; 95% CI: 0.25, 0.45; nominal p-value<0.0001. The supportive results from the non-mITT population were an estimated geometric mean ratio: 0.52; 95% CI: 0.43, 0.63); nominal p-value<0.0001(Table 8). Because the assessment of NfL as a surrogate endpoint will include data from all patients, NfL reduction was also quantified for the ITT population. In the ITT population, plasma NfL was reduced by 55% (geometric mean ratio to baseline) in the tofersen-treated participants, compared to a 12% increase in placebo-treated participants at Week 28 [difference (ratio) in geometric mean ratios for tofersen to placebo: 0.4; post hoc nominal p<0.0001] (Table 8). The NfL reduction driven by tofersen plateaued at Week 16 and was sustained at the end of treatment at Week 28 (Figure 3). Given the lack of evidence statistical significance in the primary analysis of this study and the pre-specified hierarchical

[19] Bali T, Self W, Liu J, et al. J Neurol Neurosurg Psychiatry 2017;88:99–105.
[20] Juneja T, Pericak-Vance MA, Laing NG, et al. Prognosis in familial amyotrophic lateral sclerosis: progression and survival in patients with glu100gly and ala4val mutations in Cu,Zn superoxide dismutase. Neurology 1997;48:55–7.
[21] Cudkowicz ME, McKenna-Yasek D, Sapp PE, et al. Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Ann Neurol 1997;41:210–21.

testing plan, the effect of tofersen on NfL is considered nominally statistically significant. However, the NfL reduction was consistently observed for all subgroups based on sex, disease duration since symptom onset, site of onset (i.e., limb vs. bulbar), and riluzole/edaravone use. In Study 101C, similar reductions in NfL were also observed in the CSF following tofersen treatment (difference in geometric mean ratios for tofersen to placebo of 69% and nominal $p < 0.0001$, ITT population).

Table 8: Study 101 Part C: Summary of Adjusted Geometric Mean Ratio to Baseline in Plasma NfL at Week 28

Population		Placebo	Tofersen
ITT	N Adjusted GMR to baseline Tof:plac difference in GMR (95% CI) Nominal p-value (ANCOVA+MI)	36 1.12	72 0.45 0.40 (0.33, 0.49) <0.0001
mITT	N Adjusted GMR to baseline Tof:plac difference in GMR (95% CI) Nominal p-value (ANCOVA+MI)	21 1.20	39 0.40 0.33 (0.25, 0.45) <0.0001
Non-mITT	N Adjusted GMR to baseline Tof:plac difference in GMR (95% CI) Nominal p-value (ANCOVA + MI)	15 0.95	33 0.50 0.52 (0.43, 0.63) <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.

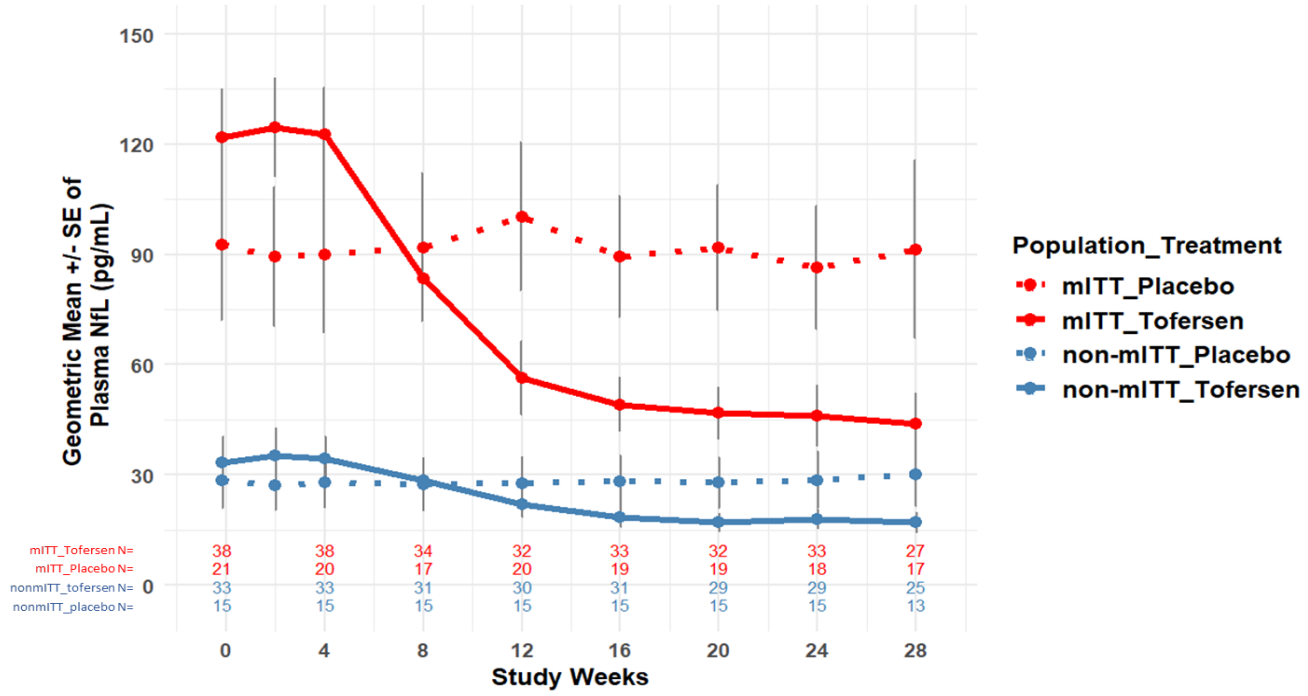
NOTE 2: Values below limit of quantitation (BLQ) are set to half of lower limit of quantitation (LLOQ, 4.9 pg/mL) in calculations.

NOTE 3: MI was used for missing data. Model included treatment, use of riluzole or edaravone, relevant baseline score and post-baseline values (natural log transformed data). Separate models for mITT and nonmITT 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.

Source: CSR Study 101 Part C, Table 23

Figure 3: Line plot of plasma NfL (pg/mL) geometric mean values +/- SE by visit (observed data) from Study 101C

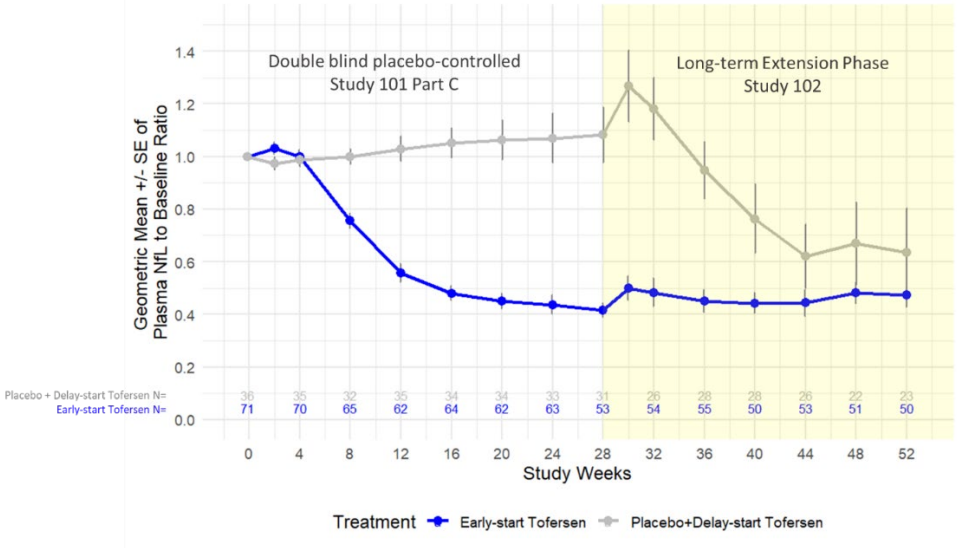


Source: Clinical Pharmacology Reviewer's Analysis

It is also of interest that notable differences were observed in baseline plasma NfL levels between the treatment arms in the mITT population. The geometric mean of baseline plasma NfL level in patients receiving placebo (93 pg/mL, SD of 94 pg/mL) was lower compared to geometric mean of baseline plasma NfL level in patients receiving tofersen (122 pg/mL, SD of 83 pg/mL) in the mITT population (Section 3.1.2.3.1 Refer to detailed discussion in Role *of Baseline NfL in explaining heterogeneity and imbalances in disease progression.*).

In the ITT population of Study 102, participants who had received tofersen in Study 101 Part C (early-start tofersen group) maintained the previously lowered plasma NfL levels following the 24 weeks of continued tofersen treatment (Figure 4). In participants in the delayed-start tofersen group (patients received placebo in Study 101 Part C), 24 weeks of treatment with open-label tofersen reduced plasma NfL levels by 44% (GMR to baseline of Study 102) in the ITT population.

Figure 4: Line plot of plasma NfL baseline to ratio geometric mean values +/- SE by visit (observed data) from Study 101 Part C and Study 102

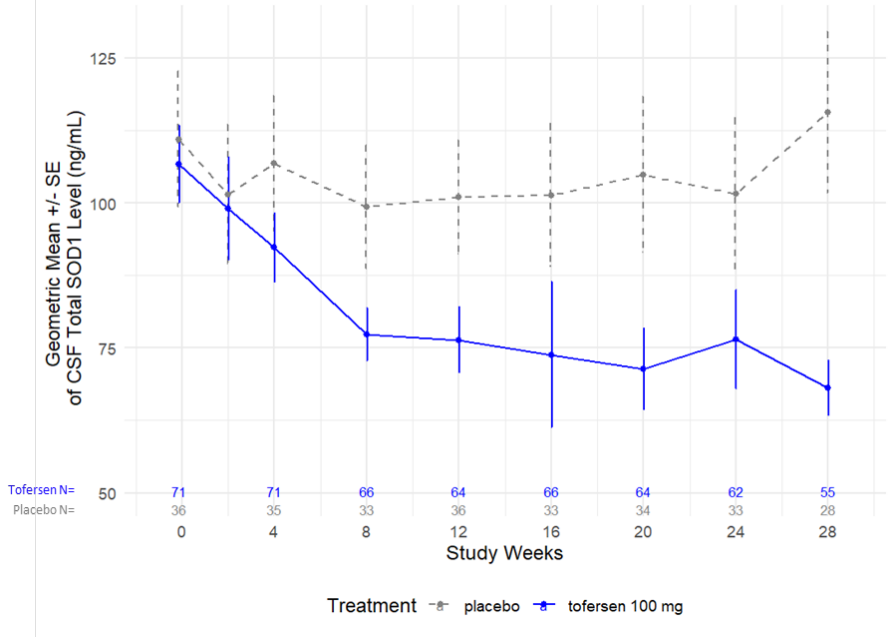


Source: Clinical Pharmacology Reviewer's analysis

Total SOD1 in CSF

In study 101C, another secondary PD endpoint was the change from baseline at Week 28 in total SOD1 concentration in CSF for the mITT population. In Study 101C, CSF was sampled before dosing at each visit in which treatment was administered (Day 1, 15, 29, and every 4 weeks thereafter) and at the final visit (4 weeks after last dose). A reduction in total CSF SOD1 protein was observed at Week 28 in the tofersen group compared to the placebo group (38% difference in geometric means ratio for tofersen to placebo, nominal $p < 0.0001$) in the mITT population. 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 $p < 0.0001$, Figure 5).

Figure 5: Line plot of total CSF SOD1 protein level (Geometric Mean±SE) by visit (observed data) from Study 101 Part C, ITT population



Source: Clinical Pharmacology Reviewer's Analysis

3.1.2.2 Assessment of Plasma NfL as a Reasonably Likely Surrogate Endpoint to support accelerated approval

The plasma NfL was evaluated as a reasonably likely surrogate endpoint to support AA for tofersen in the treatment of SOD-1ALS, based on the following aspects:

- Mechanistic evidence that plasma NfL is a biomarker that is reasonably likely to predict clinical function based on pathophysiology of SOD1-ALS and the pharmacology of tofersen
- Evidence from literature and tofersen clinical program to demonstrate the prognostic value of plasma NfL in predicting disease progression and survival in ALS.
- Evidence from tofersen clinical program to demonstrate the relationship between tofersen-driven NfL reduction and changes in clinical decline using longitudinal changes in NfL and ALSFRS-R total score, correlation analysis and causal inference analysis

Sections 3.1.2.2.1 through Sections 3.1.2.3.2 describe different aspects of the clinical pharmacology team's assessment of plasma NfL as a surrogate endpoint. Then, Section 3.1.2.4 provides summary comments on this topic, including perspectives from both the clinical pharmacology review team and the statistical review team.

3.1.2.2.1 Mechanistic Support based on Understanding of the Disease Pathophysiology and the MOA of Tofersen

The current understanding of the pathophysiology of SOD1-ALS and the pharmacology of tofersen provides mechanistic support that plasma NfL is a biomarker that is reasonably likely to predict clinical function.

In SOD1-ALS, it appears that the pathologic mutation in the SOD1 gene is closely linked to the development and clinical progression of the disease. Mutations in SOD1 gene may cause toxic accumulation of mutated or misfolded SOD1 protein.[22,23,24] Although the underlying mechanism is not fully understood, the level of misfolded SOD1 has been found to be correlated with the vulnerability of neurons in the spinal cord.[22] The release of NfL in CSF and blood is a consequence of axonal injury and the level of NfL may reflect the degree of axonal damage[5,6]. The degeneration and loss of motor neurons, the hallmarks of ALS, leads to decline in clinical function that are typically assessed by ALSFRS-R.

Tofersen is an antisense oligonucleotide targeting the mRNA for human SOD1. If tofersen does reduce neuronal injury by lowering SOD1, a reduction in NfL would be the expected outcome. Based on the pathophysiology of SOD1-ALS, this reduction in NfL could lead to slower clinical functional decline.

3.1.2.2.2 Reported Prognostic value of plasma NfL levels in ALS

The prognostic value of plasma NfL in ALS was evaluated using data from literature and ALS clinical trials.

3.1.2.2.2.1 Evidence from Literature

The Clinical Pharmacology review team conducted a meta-analysis on the prognostic value of NfL in patients with ALS to quantify the relationship between both (A) plasma NfL and disease progression slope for ALSFRS-R total score; and (B) plasma NfL and unfavorable clinical outcomes (death, tracheostomy and/or permanent assisted ventilation). Figure 6 shows the correlation between

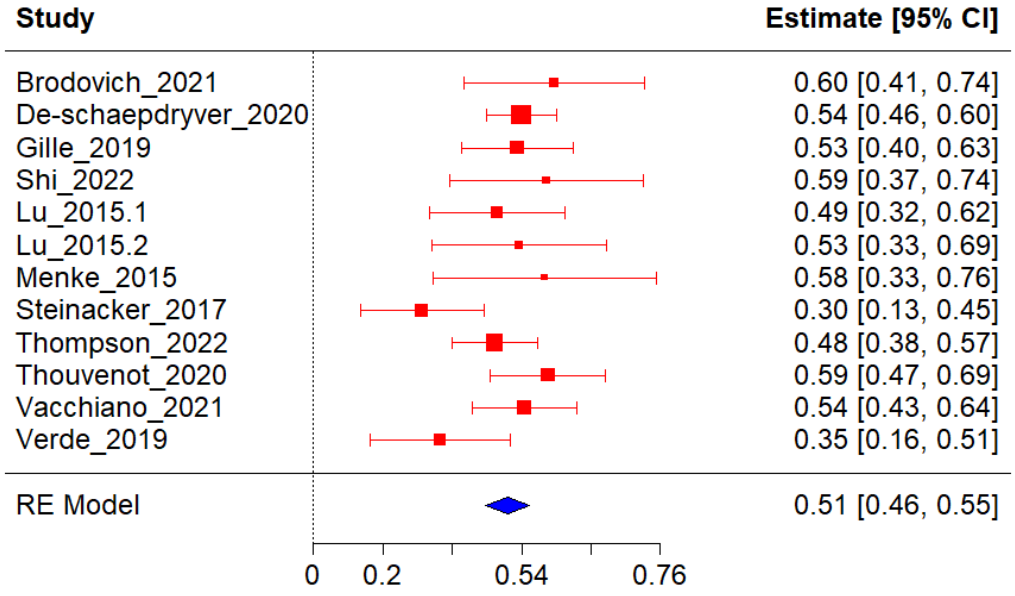
[22] Genc B, Gozutok O, Kocak N, Ozdinler PH. The Timing and Extent of Motor Neuron Vulnerability in ALS Correlates with Accumulation of Misfolded SOD1 Protein in the Cortex and in the Spinal Cord. Cells. 2020;9(2):502. Published 2020 Feb 22. doi:10.3390/cells9020502

[23] Brotherton TE, Li Y, Cooper D, et al. Localization of a toxic form of superoxide dismutase 1 protein to pathologically affected tissues in familial ALS. Proc Natl Acad Sci U S A. 2012;109(14):5505-5510. doi:10.1073/pnas.1115009109

[24] Trist BG, Genoud S, Roudeau S, et al. Altered SOD1 maturation and post-translational modification in amyotrophic lateral sclerosis spinal cord. Brain. 2022;145(9):3108-3130. doi:10.1093/brain/awac165

neurofilaments and the disease progression slope for all the published studies.[2,3,12,14,25,26,27,28,29,30,31] The overall correlation coefficient between disease progression (slope of ALSFRS-R total scores) and plasma NfL from a meta-analysis was 0.51 (95% CI: 0.46, 0.55), which suggests that higher blood NfL levels in ALS patients are associated with more rapid disease progression. We note that a large number of these studies have been published in the last three years.

Figure 6: **Forest plots showing the correlation coefficients for the relationship between disease progression slope and plasma NfL**



RE: Random Effect

Source: Clinical Pharmacology Reviewer's Analysis

[25] 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. doi:10.1136/jnnp-2019-322309

[26] Shi J, Qin X, Chang X, Wang H, Guo J, Zhang W. Neurofilament markers in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. J Cell Mol Med. 2022;26(2):583-587. doi:10.1111/jcmm.17100

[27] Menke RA, Gray E, Lu CH, et al. CSF neurofilament light chain reflects corticospinal tract degeneration in ALS. Ann Clin Transl Neurol. 2015;2(7):748-755. doi:10.1002/acn3.212

[28] 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. doi:10.1080/21678421.2016.1241279

[29] 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. doi:10.1111/ene.14063

[30] 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. Published 2021 Oct 22. doi:10.3389/fnagi.2021.753242

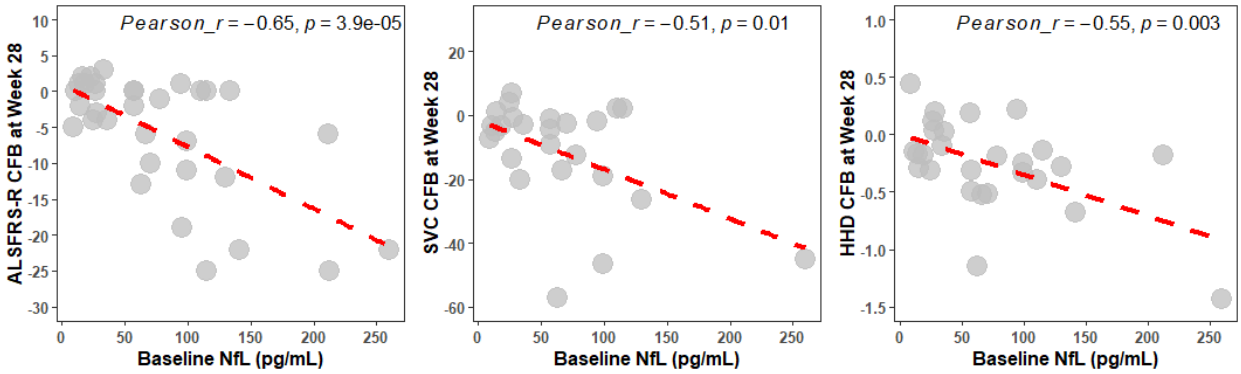
[31] Verde F, Steinacker P, Weishaupt JH, et al. Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2019;90(2):157-164. doi:10.1136/jnnp-2018-318704

The relationship between plasma NfL levels and unfavorable clinical outcomes (death, tracheostomy and/or permanent assisted ventilation) was quantified using multivariate cox-regression in several research studies. [29,30, 32] The hazard ratios for plasma NfL obtained from literature were used to calculate relative hazard risk which suggested that patients with higher plasma NfL had a higher risk of unfavorable clinical outcomes in all three studies . Other studies have also reported shortened survival for subjects with higher plasma NfL levels.[26] Overall, the literature supports that higher levels of neurofilament are associated with a higher risk of unfavorable clinical outcomes.

3.1.2.2.2.2 Evidence from Placebo Arm in Tofersen Clinical Program

As part of the evaluation for use of NfL as a prognostic biomarker in ALS, the placebo data (n=33) from Study 101 C was analyzed to identify prognostic factors that were associated with change from baseline in clinical endpoints (ALSFRS-R total score, SVC, and HHD megascore) at Week 28. Figure 7: shows the correlation between baseline plasma NfL and change from baseline in clinical endpoints at Week 28 in all study completers from the placebo group. This finding demonstrates that placebo subjects with higher baseline NfL experienced more decline across all clinical endpoints at Week 28, which is consistent with the meta-analysis findings.

Figure 7: Correlation between Baseline Plasma NfL and Clinical Endpoint Change from Baseline at Week 28 in Study Completers from Placebo Group



Source: Clinical Pharmacology Reviewer's Analysis

Regression analysis was performed to identify the presence of additional prognostic factors other than plasma NfL that can affect ALSFRS-R total score at Week 28. Table 9 provides a list of prognostics factors and various neurofilament metrics used in the analysis dataset. These variables were selected based on their availability in the dataset as well as potential clinical relevance.

[32]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. doi:10.1212/WNL.0000000000009559

Table 9: List of Demographics, Disease Characteristics, and Various Neurofilament Metrics Used in the Analyses

A. List of potential prognostic factors		
Demographics	**Disease Characteristics**	
Age	*ALSFRS-R total score*	*Time from symptom onset*
Sex	*ALSFRS-R slope*	*Site of Onset*
Weight	*Plasma NfL*	*Edaravone or Riluzole use*
Height	*Plasma pNfH*	*SOD-1 protein*
BMI	*Slow Vital Capacity (SVC)*	
B. Various metrics of Neurofilaments explored		
Baseline NfL (pg/mL)	*NfL-time slope*	
NfL change (pg/mL)	*Log NfL (pg/mL)*	
NfL change (%)	*Log daily area under NfL-time curve (pg/mL)*	
NfL ratio to baseline	*Log linear-model-estimated area under NfL-time curve until Week 28 (pg.day/mL)*	

Source: Clinical Pharmacology Reviewer's Analysis

Two regression methods were used: linear regression and lasso regression. The findings from both analyses suggested that baseline levels of plasma NfL were a significant predictor (p-value<0.001) for ALSFRS-R CFB at Week 28, even after adjusting for multiple potential baseline prognostic factors and various transformations of NfL metrics. These analyses may be affected by the limited sample size (n=33). However, these results, along with the meta-analysis of the literature data outlined above, support the prognostic value of plasma NfL in ALS.

3.1.2.2.3 Relationship between Reduction in Plasma NfL and Clinical Endpoint

Considering the prognostic value of plasma NfL levels in ALS, further analyses were conducted to evaluate the relationship between plasma NfL reduction with tofersen treatment and reduction in clinical decline.

3.1.2.2.3.1 Longitudinal Changes in Plasma NfL and ALSFRS-R

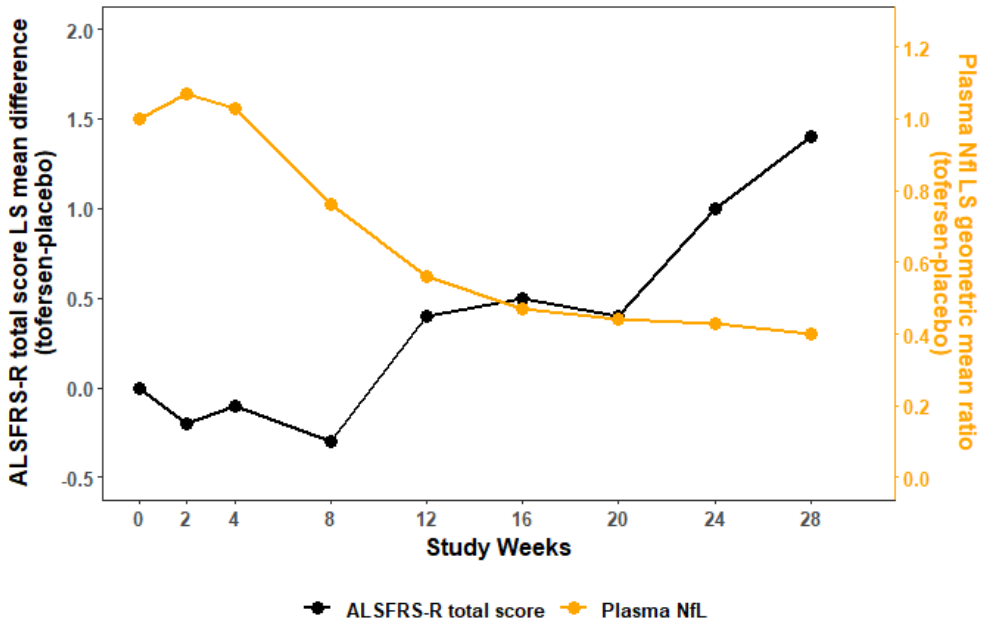
Longitudinal changes in plasma NfL and ALSFRS-R suggested a potential relationship between the reductions in plasma NfL in the tofersen treatment group and reduction in decline of clinical endpoints.

This analysis focused on the entire (ITT) data set so as to provide the largest number of patients, and broadest range of NfL changes and ALSFRS-R changes to assess this relationship. Data from the ITT population (N=108) of Study 101 C show that the mean NfL reductions in the tofersen treatment group appear to start from Week 4 and reach their maximum effect as early as Week 16. Beyond Week 16, the mean reductions in plasma NfL are relatively consistent with those at Week 16. Although the changes in ALSFRS-R in the tofersen group relative to the placebo group were not statistically significant through Week 28, numerical differences were observed after Week 8 and continued through Week 28. (

Figure 8). This could indicate that a treatment effect of slowing of disease progression may not become apparent until several weeks after treatment initiation.

To further understand the relationship between NfL reductions in the tofersen treatment group and a reduction in clinical decline, two analyses were subsequently conducted: (i) correlation analysis between plasma NfL reduction at Week 28 and ALSFRS-R CFB at Week 28; (ii) causal inference analysis to quantify the impact of reductions in plasma NfL in the tofersen group at Week 16 on reduction in clinical decline of ALSFRS-R total score at Week 28. Given that mean plasma NfL values are consistent from Week 16 to Week 28, we expected that using NfL at Week 16 or Week 28 in the analyses should provide similar results.

Figure 8: Line plots of ALSFRS-R total score LS mean difference (tofersen-placebo) and Plasma NfL LS mean ratio to baseline (tofersen-placebo) by visits



Note: For least squares mean calculation on ALSFRS-R total score, treatment is included as a fixed effect after adjusting for baseline disease duration since symptom onset, baseline ALSFRS-R total score, and use of riluzole or edaravone.
For least squares mean calculation on NfL, treatment is included as a fixed effect after adjusting for baseline disease duration since symptom onset, log baseline NfL, and use of riluzole or edaravone.
Source: Adapted from Clinical study report of Study 101 Part C

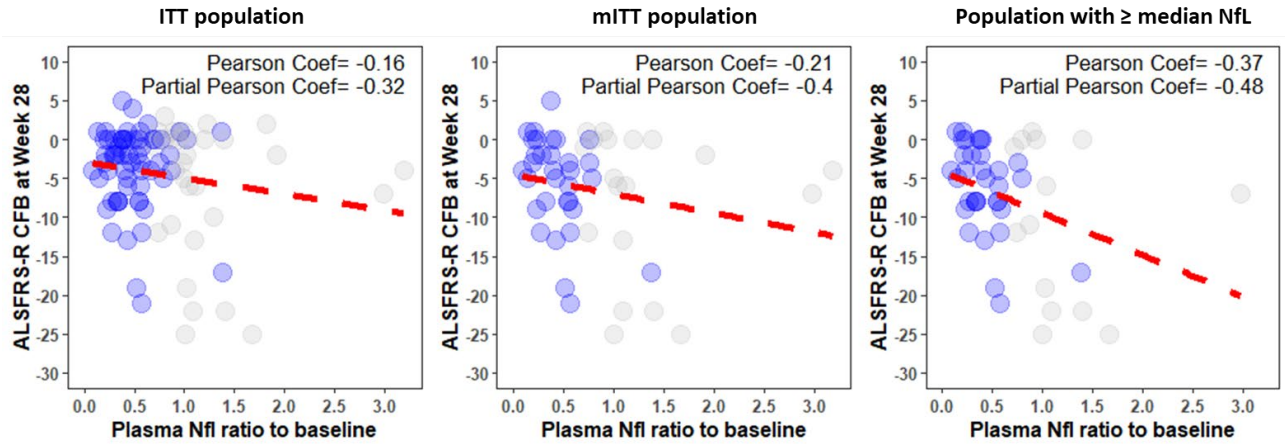
3.1.2.2.3.2 Correlation analysis

Correlation analysis demonstrated a relationship between reduction in NfL and ALSFRS-R CFB at Week 28. Figure 9A shows the relationship between plasma NfL reduction at Week 28 and ALSFRS-R CFB at Week 28 for the ITT population and in the mITT population (Figure 9A). In addition a subgroup with baseline levels of NfL ≥ the median was also evaluated. Correlation coefficients are provided with and without adjustment for other baseline prognostic variables. The prognostic variables were selected based on the findings from the regression analysis of tofersen clinical data and clinical relevance. The selected variables for this analysis include: baseline NfL, baseline % predicted SVC, time since symptom onset, sex, and weight. The results demonstrated that plasma NfL reduction was associated with reduction in clinical function decline of ALSFRS-R total score in both the ITT and mITT populations and in the higher median baseline subgroup. The impact of NfL reduction on ALSFRS-R CFB at Week 28 was most prominent in the high baseline subgroup, as might be expected because higher baseline NfL predicts more rapid progression such that a treatment benefit, if present, may have been more apparent.

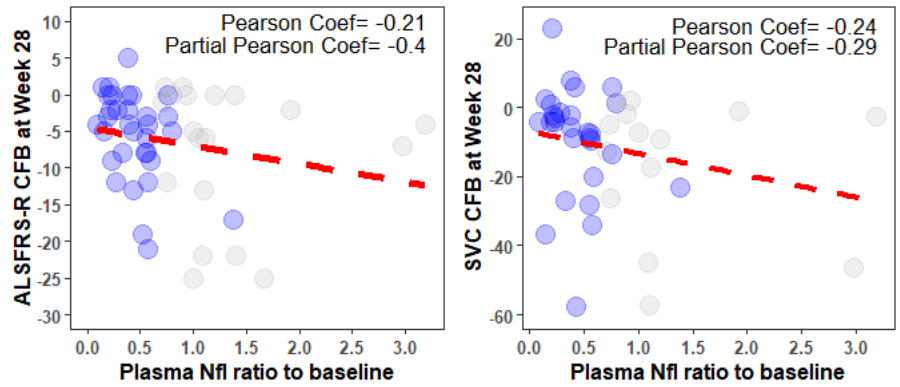
The impact of different clinical endpoints or NfL metrics on the correlations were evaluated using mITT population. Similar correlations were observed in the mITT population between plasma NfL and other clinical endpoints such as SVC (% predicted) at Week 28 (Figure 9B). These findings were consistent not only for NfL ratio to baseline, but also for other plasma NfL reduction metrics, such as percent reduction NfL, change from baseline in NfL, and absolute plasma NfL levels at Week 28 (Figure 9C).

Figure 9: Correlation analysis of plasma NfL reduction with ALSFRS-R score CFB at Week 28 across different population (A), clinical endpoints (B) and plasma NfL reduction metrics (C)

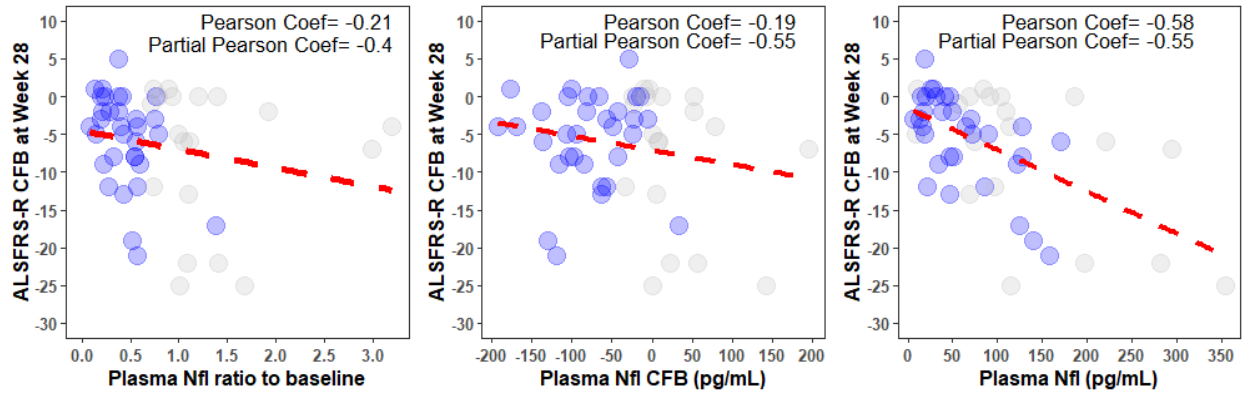
A. Impact of different populations



B. Impact on different clinical endpoints (mITT population)



C. Impact of different Plasma NfL reduction metrics (mITT population)



ITT: Intention-to-treat; mITT: modified ITT; mITT criteria: ALSFRS-R pre-randomization slope (>0.2 - 0.9/month) and SOD1 mutation type; Partial Pearson correlation adjusted for NfL levels, SVC sex, time since symptom onset and weight; Blue and grey circles represent subjects from Treatment and Placebo group; Correlations in B. and C. are based on mITT population

Source: Clinical Pharmacology Reviewer's Analysis

Correlation analyses suggest that plasma NfL reduction appears to be associated with reduction in clinical decline across clinical endpoints, including ALSFRS-R at Week 28.

The mITT population was intended to select the subjects with faster disease progression; however, baseline imbalances in NfL were observed, and may be relevant given the strength of this covariate as a predictor of disease progression. A causal inference model was developed to assess progression considering differences in baseline NfL and other baseline characteristics by constructing a model-based matched control for each tofersen-treated participant. The intent of this model-derived matched control was to attempt to predict disease progression of patients in the treatment arm as if they had received placebo, so as to better assess the effect of tofersen, considering differences in expected rates of progression. These analyses must be considered as exploratory, given their post-hoc nature and the limited size of the placebo group.

3.1.2.2.3.3 Causal Inference Analysis

The objective of the causal inference analysis was to evaluate the relationship between reduction in plasma NfL with tofersen at Day 113 (Week 16) and changes in clinical outcome measures (ALSFRS-R total score, percent predicted SVC, HHD megascore, ALSDAQ-5 total score, and EQ-5D-5L utility score) at Day 197 (Week 28). Data from ITT population (N=108) of Study 101C was used. The model partitioned the effect of tofersen on the change from baseline in the clinical endpoint at Week 28 into three components: (i) natural disease progression, (ii) drug effect via NfL pathway, and (iii) drug effect via non-NfL pathway. Please refer to Appendix 5.1 for the model structure equations.

For ALSFRS-R total score, the model suggests that, at mean baseline NfL level of 96.78 pg/mL, every 10 pg/mL reduction in plasma NfL at Week 16 is associated with an average of 0.8 points reduction (p=0.0038) in worsening on ALSFRS-R at Week 28 (Figure 10,

Table 10). The p-value assesses the strength of the relationship between plasma NfL reduction with tofersen treatment and ALSFRS-R CFB at Week 28 when adjusted for disease progression and drug effect via non-NfL pathways (with these components assessed based upon changes from baseline in the placebo group). Note that this is not a p-value for the treatment effect for the Study 101C (which was not statistically significant). Similar trends in the relationship of the reduction in plasma NfL with tofersen treatment were observed for other clinical endpoints, including percent predicted SVC, HHD megascores, ALSDAQ-5 total score, and EQ-5D-5L utility score (

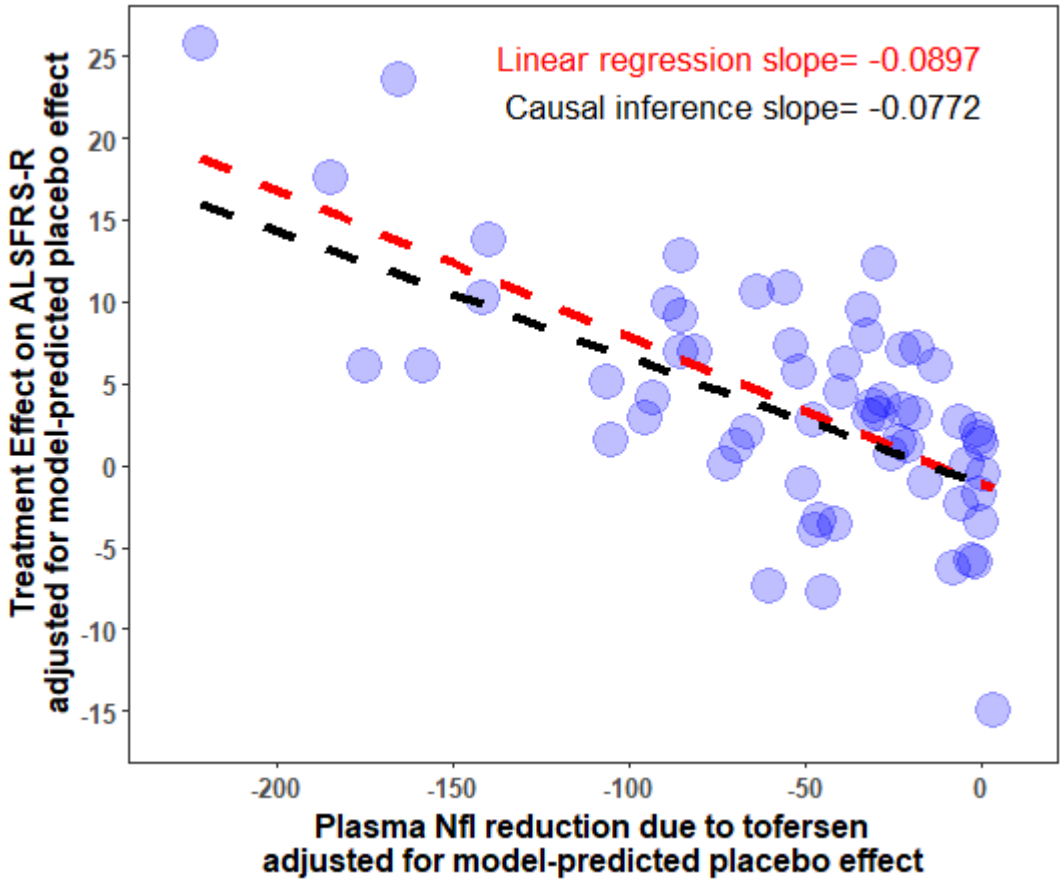
Table 10).

Table 10: Reduction in Worsening with Tofersen per Unit of NfL reduction at the Sample Mean Baseline NfL of 96.78 pg/mL across all Clinical Outcome Measures

Clinical outcome measure	Reduction in worsening with tofersen (vs. untreated) per 1 unit of NfL lowering at sample mean baseline NfL (96.78 pg/mL)
ALSFRS-R total score	0.0772 (p=0.0038)
SVC (percent-predicted)	0.1451 (p=0.0706)
HHD overall megascore	0.0029 (p=0.1303)
ALSAQ-5 total score	0.2194 (p=0.0056)
EQ-5D-5L utility score	0.0017 (p=0.0894)

1 unit of NfL corresponds to 1 pg/mL of NfL
Source: Section 2.7.2 Summary of Clinical Pharmacology studies, page 65, table 9

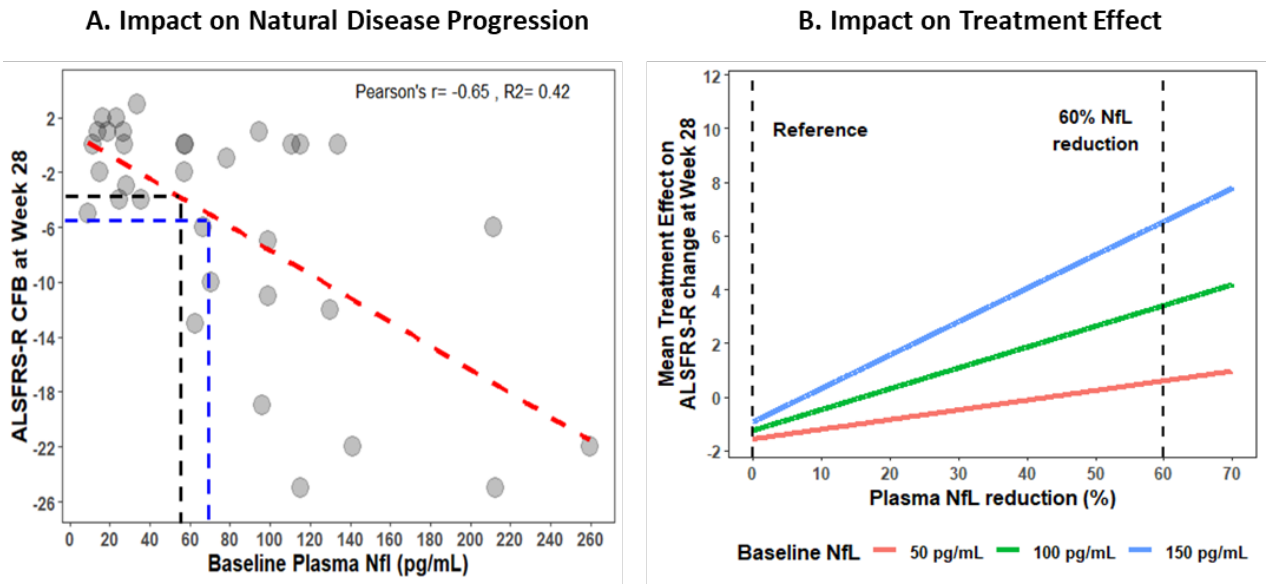
Figure 10: Relationship between Plasma NfL reduction due to tofersen and treatment effect on ALSFRS-R changes from baseline after adjusting for natural ALSFRS-R and NfL progression in tofersen-treated subjects



Source: Clinical Pharmacology Reviewer's Analysis

An advantage of the causal model is that it incorporates potential imbalances in influential baseline characteristics between the placebo group and the treatment group. One apparent difference between the treatment groups appears to be the baseline plasma NfL. The mean baseline plasma NfL levels in the treatment group were 10 pg/mL higher than Placebo group (Placebo: 57 pg/mL and Treatment: 67 pg/mL) in the ITT population. The impact of differences in baseline NfL on the natural disease progression is shown using placebo data (Figure 11A), which suggests that the enrolled patients in the tofersen treatment group might have had greater disease progression than did the placebo group without treatment. The causal model accounts for the difference in baseline NfL by predicting the NfL level and ALSFRS-R through the trajectory of the natural disease progression seen in the placebo group. The model predicts that if tofersen-treated subjects in Study 101C (n=72) were randomized to placebo, they would have experienced faster disease progression with an average of 3.83 points more decline on ALSFRS-R over 28 weeks as compared to the patients randomized to placebo.

Figure 11: Impact of baseline NfL on the (A) disease progression (ALSFRS-R change at Week 28); and (B) the Relationship between Plasma NfL reduction and Treatment Effect on ALSFRS change at Week 28



Plot A: Red dashed line represents linear regressed line. Black and blue dashed line represents ALSFRS changes at Week 28 at geometric mean baseline NfL of Placebo and Treatment group from ITT population respectively
Source: Clinical Pharmacology Reviewer's analysis

These analyses suggest that the effect of plasma NfL reduction on ALSFRS-R CFB at Week 28 can be affected by baseline NfL, as shown by using the causal inference model (Figure 11 B). The model was used to predict tofersen effect at Week 28 in three typical subjects with baseline NfL levels of 50 pg/mL, 100 pg/mL, and 150 pg/mL with varying degree of plasma NfL reduction. Overall, the results showed that reductions in plasma NfL with tofersen administration are associated with less decline in clinical

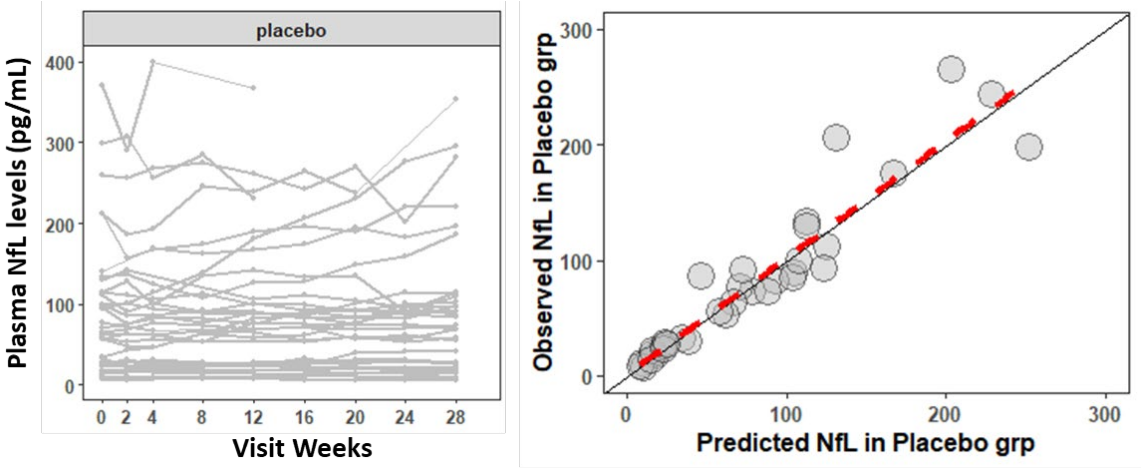
function, or slowing of disease progression and that this relationship is more pronounced in subjects with higher baseline NfL levels.
The limitations of this analysis must be recognized including the post-hoc nature (with post-hoc selection of key covariates in the model), and the small size of the placebo group upon which the natural progression was assessed. In addition, although a small size study, this was a randomized comparison, so "correcting" for a post-hoc imbalance (here plasma NfL) must be considered with caution.

As noted already, the focus of the analysis was to provide the trend between NfL reduction and reduction in ALSFRS-R decline. The model has some assumptions which could influence the results. Additional analyses were performed to evaluate some of these assumptions and are discussed below.

1. *Discussion on residual error model for NfL natural progression in Treatment Arm:* The NfL natural progression in the treatment arm was predicted based on the placebo data and does not add a residual error term in the model. Observed data suggest that, for most individuals, plasma NfL levels at Week 16 is almost the same as their baseline NfL (Figure 12A). The predicted NfL, following the exponential transformation back to original scale, in the placebo arm without residual error well describe the observed NfL levels at Week 16 in the placebo group (Figure 12B). Therefore, the impact of no residual error term in the NfL natural progression model for the treatment group is expected to be low, and unlikely to affect estimate for NfL reduction effect on clinical decline. To further confirm this hypothesis, we introduced the residual error component of NfL progression in the model, which showed that the median estimate (-0.085, n= 1000 simulations) for NfL effect was, in fact, approximately 8% steeper (better) than the parameter estimated from model with no residual error component of NfL progression (Figure 12C).

Figure 12: (A) Longitudinal changes in Plasma NfL levels up to Week 28; (B) Model diagnostic for plasma NfL in the Placebo Group; and (C) Distribution of the NfL reduction effect (or, slope) on ALSFRS-R CFB at Week 28

A. **B.**



C.

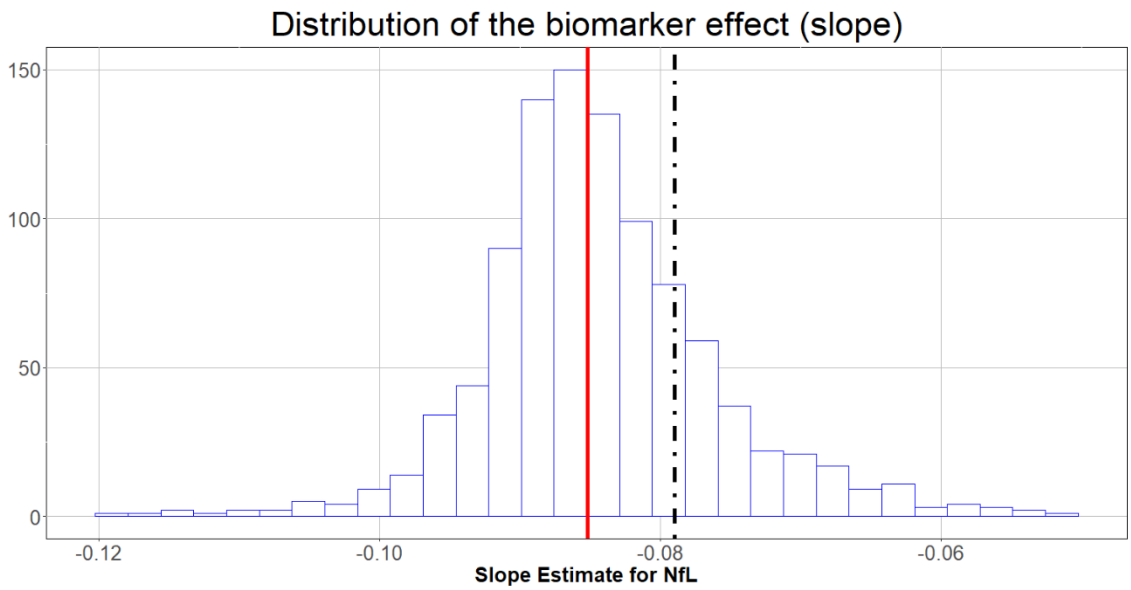
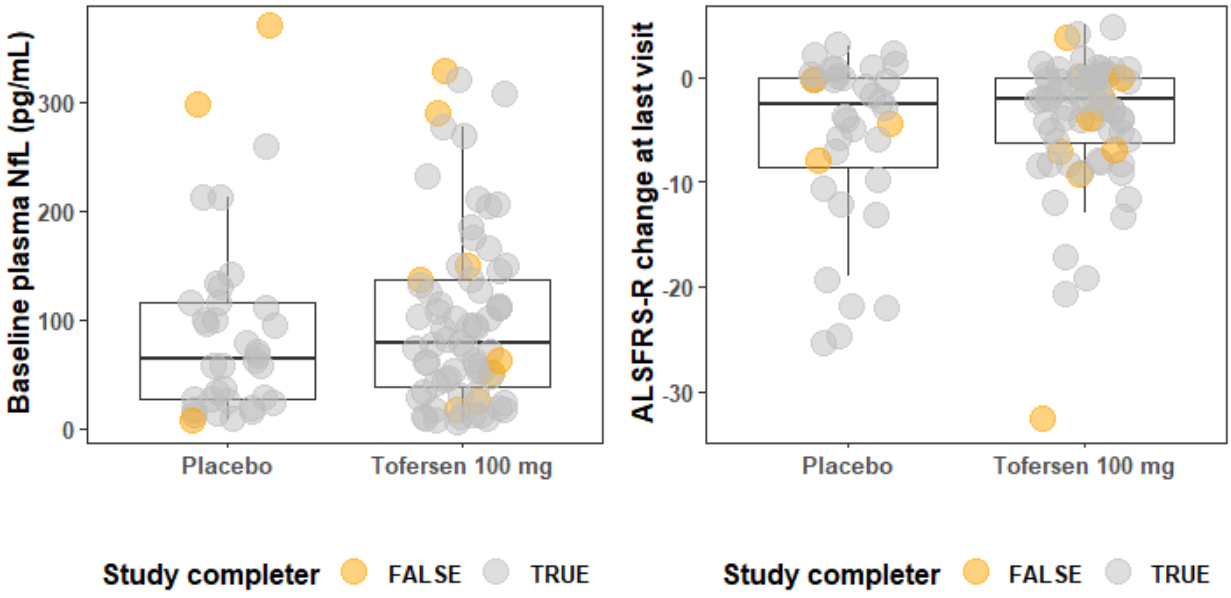


Figure C: Red line is median slope estimated from 1000 simulations with residual error component for NfL added in the model; while the black dashed line is a parameter with no residual error component for NfL added in the model

Source: Clinical Pharmacology Reviewer's analysis

2. *Discussion on Analysis based on Study Completers:* The analysis was based on study completers, accounting for approximately 90% of the enrolled patients, with the assumption of missing completely at random. The study non-completers (12 out of 108; Treatment: 9 subjects; Placebo: 3 subjects) included one death in tofersen group. Out of these 12 subjects, 7 subjects were enrolled for at least Week 16. The study-completers do not appear to be apparent outliers across treatment arms as they have similar values of baseline NFL and ALSFRS-R changes at the last visit prior to their discontinuation (Figure 13). Of note, exclusion of two placebo-treated study-noncompleters with high baseline NfL (>260 pg/mL) in the analysis is unlikely to affect the treatment effect estimation as the tofersen group has its own matched control. Overall, the impact of these missing data on the relationship between NfL reduction and ALSFRS-R decline is expected to be low.

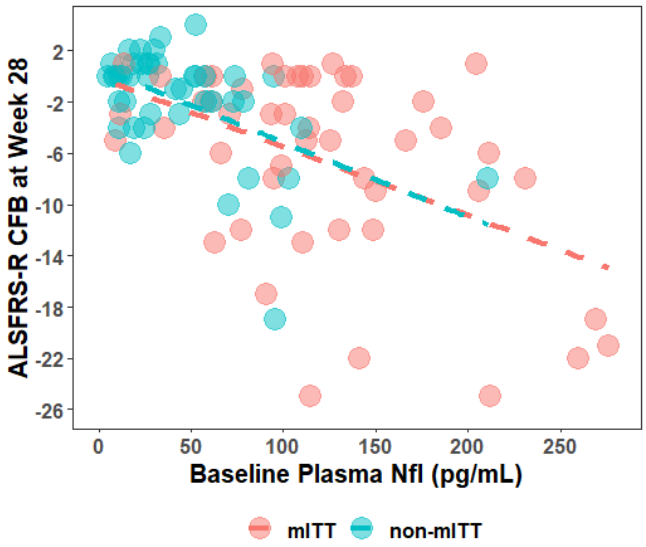
Figure 13 Comparison of baseline NfL levels and ALSFRS-R total scores at last visit across Treatment and Placebo group



Source: Clinical Pharmacology Reviewer's analysis

3. *Selection of ITT population for the analysis:* The analysis was performed on the ITT population to maximize the information from the trial (n=108); this population was not narrowed by any selection criteria such as mITT or baseline NfL. Subgroup analysis using mITT /non-mITT population was not pursued because it would have notably shortened the range of key study variables including disease progression slope, baseline NfL, and ALSFRS-R change, such that identifying a relationship in a smaller number of patients with a narrower range of key variables would be more challenging. For instance, the subjects from non-mITT population (n=48) included lower baseline NfL, while mITT population (n=60) included subjects with higher baseline NfL (Figure 14). These differences in baseline NfL would impact both disease progression as well as treatment effect, and thus would give biased estimate for plasma NfL reduction effect. Therefore, the ITT population was concluded to be more appropriate to estimate plasma NfL reduction effect on clinical endpoint.

Figure 14: Correlation between Baseline Plasma NfL and ALSFRS-R Change from Baseline at Week 28 colored by population



Source: Clinical Pharmacology Reviewer's analysis

4. *Selection of plasma NfL reduction at Week 16:* As the model assesses the predictive nature of plasma NfL, Week 16 was selected as it was the earliest time at which plasma NfL was largely reduced and stabilized (

Figure 8). Sensitivity analyses were also performed at other study visits such as Week 12, 16, 20, 24 or 28 to evaluate its impact on the estimation of drug effect via NfL pathway. The results suggested that impact of the reduction in plasma NfL on reduction in clinical decline of ALSFRS-R CFB at Week 28 was consistent across Weeks 16, 20, 24 or 28 (Table 11).

Table 11: Study 233AS101 Part C: Relationship Between Reduction in Plasma NfL due to Tofersen and Reduction in Worsening in ALSFRS-R Total Score

Timepoint Used for Plasma NfL Value	Reduction in worsening with tofersen (vs. untreated) per 10 unit of NfL lowering at sample mean baseline NfL (96.78 pg/mL)	Treatment Effect favoring tofersen
Week 12	0.361 (p=0.1149)	4.08
Week 16	0.772 (p=0.0038)	3.83
Week 20	1.075 (p<0.0001)	3.82
Week 24	0.789 (p<0.0001)	3.65
Week 28	0.733 (p=0.0003)	3.89

Source: Adapted from the Response to FDA Information Request dated January 17, 2023

5. *Adequacy of the covariate selection:* Covariate selection is important as some potential prognostic variables may be correlated with NfL and can lead to overestimation of NfL effect, if ignored. The applicant model has included baseline covariates such as age, NfL, ALSFRS-R total score, SVC, and ALSFRS-R decline slope for ALSFRS-R model based on their potential prognostic effect on disease progression. Analyses were performed to evaluate the adequacy of the selected covariates. Briefly, exploratory graphical analysis was first conducted to identify baseline variables that may have impact on ALSFRS-R progression, which screened ten baseline variables: ALSFRS-R total score, ALSFRS-R decline slope, percent predicted SVC, NfL levels, time since ALS diagnosis, mutation effect, edaravone or riluzole use, age, sex, and weight. All these covariates were explored as covariates in the model. Inclusion of additional prognostic variables did not improve model performance, and the covariates selected in the applicant's model were reasonable. It was observed that NfL reduction at Week 16 contributes to reduction in clinical decline for ALSFRS-R total score in all explored models, and not just the final selected model.

To conclude, the causal inference model provides evidence that the extent of reduction in NfL with treatment can predict the extent of clinical outcome.

3.1.2.3 Evaluation of NfL's Prognostic Value based on Study 101C and Study 102 results

Based on the above findings about the value of plasma NfL in predicting disease progression, the review team conducted additional analyses to evaluate the impact of baseline NfL on disease progression in patients in Study 101C and subsequent evaluation of results from Study 102.

This further evaluation focused on: a) the use of baseline NFL as a prognostic marker for characterization of disease progression, b) evaluation of the enrichment criteria to define a rapidly progressing population (mITT population), and c) evaluation of the long-term treatment effect of tofersen by comparing the data from patients originally randomized to placebo (tofersen early start) to patients originally randomized to placebo (delayed start group) at Week 52.

3.1.2.3.1 Role of Baseline NfL in explaining heterogeneity and imbalances in disease progression.

ALS disease progression varies substantially across SOD1 mutation types based on literature findings and the observations from the Study 101C (i.e., there was a range of -4.2 to 0.86 points/month decline in the ALSFRS-R among those patients in the placebo group). Disease heterogeneity presents unique challenges and uncertainties for evaluating therapeutics in clinical trials of ALS.[33] To enrich a clinical trial with patients who are expected to progress to advanced disease more rapidly, the literature has reported predictive approaches based on baseline variables, including time from symptom onset to baseline, the ALSFRS-R score at baseline, and the slope of the ALSFRS-R score at baseline (calculated using a score of 48 the day prior to the day of symptom onset, also known as pre-randomization

[33] Goyal NA, Berry JD, Windebank A, et al. Addressing heterogeneity in amyotrophic lateral sclerosis CLINICAL TRIALS. Muscle Nerve. 2020;62(2):156-166. doi:10.1002/mus.26801

slope).[34] To predict survival time, different predictors including progression rate (based on ALSFRSR-R change), bulbar versus non-bulbar onset, diagnostic delay, etc. were reported.[35] In recent years, biomarkers such as NFs, CHIT1, CHI3L1 were also reported to be associated with disease progression and survival, and thus offer potential as prognostic factors.[36,37,38] Among these biomarkers, NfL was consistently demonstrated to correlate with rate of disease progression in patients living with ALS and has been regarded as a promising prognostic biomarker to aid patient stratification in clinical trials.[39]

In Study 101C, the mITT population (fast progressors) were selected with enrichment criteria based on pre-randomization ALSFRS-R slope, SOD1 mutation types, and an SVC cut-off value. NfL baseline level was not considered for patient enrichment at the time of study design, given that much of what is known about NfL has been reported since design and initiation of the pivotal study. The review team conducted analyses to compare the prognostic value between using pre-randomization ALSFRS-R slope and plasma NfL baseline based on data from Study 101C.

Among the SOD1-ALS patients in the placebo group from study 101C, baseline plasma NfL was found to provide stronger correlation with progression of ALSFRS-R measured as either change from baseline to Week 28 (N=33) or ALSFRS-R slope over 28 weeks (post-randomization slope, N=36) than pre-randomization slope of ALSFRS-R score (slope between symptom onset and randomization) (Figure 15 and Figure 16).

[34] Taylor AA, Fournier C, Polak M, et al. Predicting disease progression in amyotrophic lateral sclerosis. Ann Clin Transl Neurol. 2016;3(11):866-875. Published 2016 Sep 7. doi:10.1002/acn3.348

[35] Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol. 2018;17(5):423-433. doi:10.1016/S1474-4422(18)30089-9

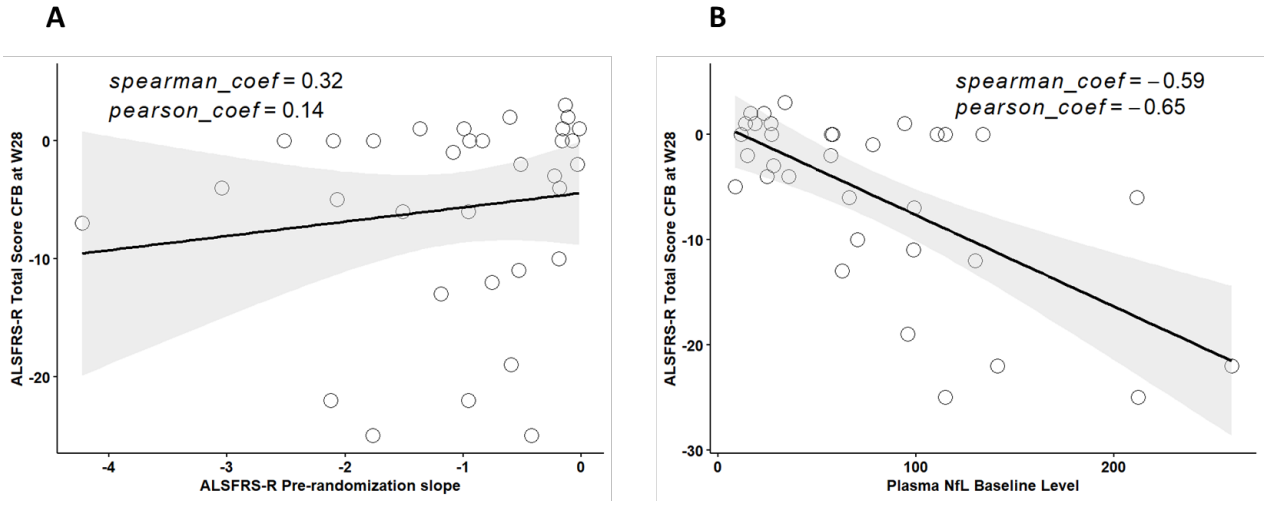
[36] Thompson AG, Gray E, Bampton A, Raciborska D, Talbot K, Turner MR. CSF chitinase proteins in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2019;90(11):1215-1220. doi:10.1136/jnnp-2019-320442

[37] Masrori P, De Schaepdryver M, Floeter MK, et al. Prognostic relationship of neurofilaments, CHIT1, YKL-40 and MCP-1 in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2022;93(6):681-682. doi:10.1136/jnnp-2021-327877

[38] Gaur N, Perner C, Witte OW, Grosskreutz J. The Chitinases as Biomarkers for Amyotrophic Lateral Sclerosis: Signals From the CNS and Beyond. Front Neurol. 2020;11:377. Published 2020 May 27. doi:10.3389/fneur.2020.00377

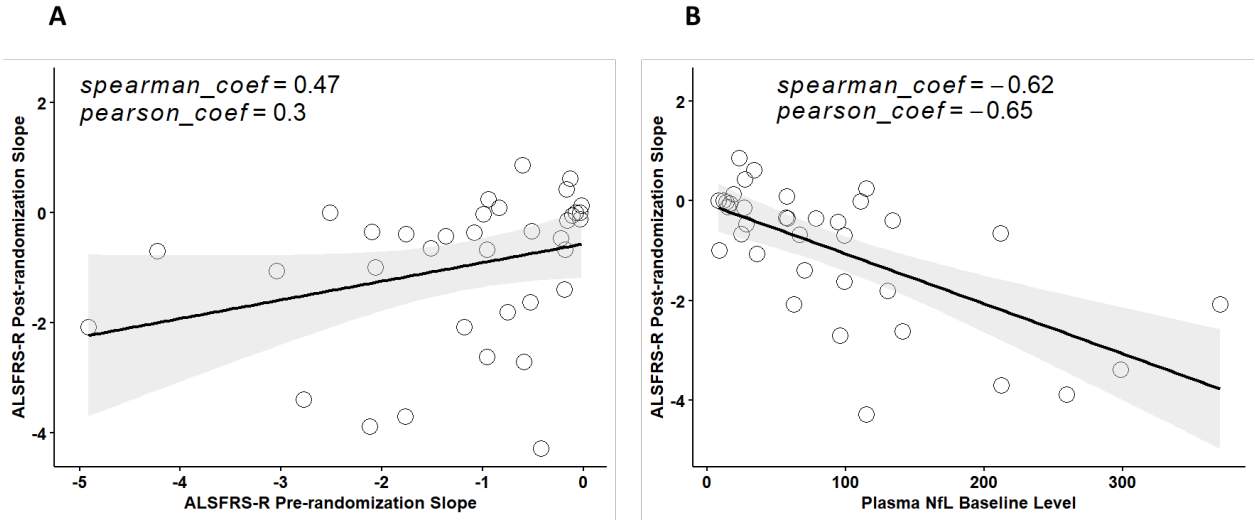
[39] Dreger M, Steinbach R, Otto M, Turner MR, Grosskreutz J. Cerebrospinal fluid biomarkers of disease activity and progression in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2022;93(4):422-435. doi:10.1136/jnnp-2021-327503

Figure 15 Correlation Analysis of (A) pre-randomization slope of ALSFRS-R score or (B) NfL baseline with ALSFRS-R total score change from baseline (CFB) at Week 28 in Study 101C



Note: Solid line as regression line and shaded area represents 95% confidence interval
Source: Clinical Pharmacology Reviewer's Analysis

Figure 16: Correlation Analysis of (A) pre-randomization slope of ALSFRS-R score or (B) NfL baseline with post-randomization slope of ALSFRS-R total score in Study 101C



Note: Solid line as regression line and shaded area represents 95% confidence interval
Source: Clinical Pharmacology Reviewer's Analysis

Compared to pre-randomization slope, baseline plasma NfL correlates better with the ALSFRS-R CFB at Week 28 and has acceptable within-subject variability when a single measurement at baseline was considered from predicting the disease progression. Therefore, baseline plasma NfL appears to offer better prognostic value than pre-randomization slope in predicting ALSFRS-R change in SOD1-ALS patients from Study 101C.

Given the prominence of baseline plasma NfL as a prognostic factor, it is also noteworthy that baseline NfL levels in the mITT population showed a trend of higher values in the tofersen group (25^{th}- 75^{th}

percentile: 94-183 pg/mL) compared to placebo group (25^{th}- 75^{th} percentile: 63-141 pg/mL). The geometric mean baseline plasma NfL was approximately 29 pg/mL (23%) higher in the tofersen group (122 ± 83 (SD) pg/mL) compared to placebo (93 ± 94 (SD) pg/mL). Because higher NfL baseline level is associated with faster disease progression rate in ALS, the trend of higher NfL baseline may imply a risk for faster disease progression in the tofersen treatment arm compared to patients in the placebo group. As predicted by the causal inference analyses, participants randomized to the tofersen group in the ITT population if they had not received treatment, would be expected to have had more decline over 28 weeks compared to the decline that was actually observed in placebo group. (Refer to section 3.1.2.2.3.3 Causal Inference Analysis). This finding suggests that the imbalance of baseline plasma NfL in the mITT population may have influenced the study results.

Because the pre-specified primary clinical endpoint was evaluated in the modified intent-to-treat (mITT) population, the selection of "fast progressors" to define the mITT population may not have been the optimal variable on which to base this population selection that was intended to identify individuals at risk of more rapid functional loss and also to reduce the heterogeneity of rates of progression, enhancing the ability to detect a treatment effect on ALSFRS-R.

The mITT population consisted of the subset (n=60) of participants who met the prognostic enrichment criteria for rapid disease progression based on their SOD1 mutation type and pre-randomization ALSFRS-R slope (also referred to as the enriched or faster progressing/faster progressor subgroup, Table 12).

Table 12: Protocol-Defined Disease Progression Subgroups

	Faster-Progressing Subgroup ("enriched"; mITT)	**Slower-Progressing Subgroup ("other"; non-mITT)**
Mutation type and pre-randomization ALSFRS-R slope	Protocol-defined *SOD1* mutation historically associated with shorter survival[a] and ≥ 0.2 points/month pre-randomization slope OR Another *SOD1* mutation and **≥** 0.9 points/month pre-randomization slope	Another *SOD1* mutation and < 0.9 points/month pre-randomization 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
Source: Summary of Clinical Efficacy, Table 2

It was noted that the placebo group from the enriched population (mITT) had a very variable post-randomization slope of ALSFRS-R total score ranging from -4.3 to 0.24 points/month. In addition, it was

noted that six patients (29%) from the enriched population (mITT) receiving placebo had no change or saw an increase of ALSFRS-R total score (improvement) at Week 28 compared to baseline.

When evaluating the disease progression rate within a pre-specified SOD1 mutation, a wide range was also noted for A5V carriers with a pre-randomization slope ranging from 0.4-5.3 points/month (N=19, placebo and tofersen groups) and the in-study slope ranging from 0.3-4.2 points/month (N=6, placebo group only). These data suggested that the heterogeneity of the disease progression rate was not well-controlled in the mITT population when relying on mutation type and pre-randomization slope.

In addition, the mITT population appeared to have slower rate of decline in ALSFRS-R during the study comparing to what was predicted or expected based on the pre-randomization slope. The study design anticipated a slope of decline of -3.83 per month for the placebo participants (i.e., 24.7-point decline over 28 weeks). The observed mean pre-randomization slope was -1.8 (SD of 1.2) and -1.7 (SD of 1.6) for placebo and tofersen group, respectively. Comparing the in-study slope (rate of decline from Day 1 to Day 197 after randomization) to the pre-randomization slope in placebo group, both the mITT and ITT populations had 22% and 17% slower observed slope in-study, respectively.

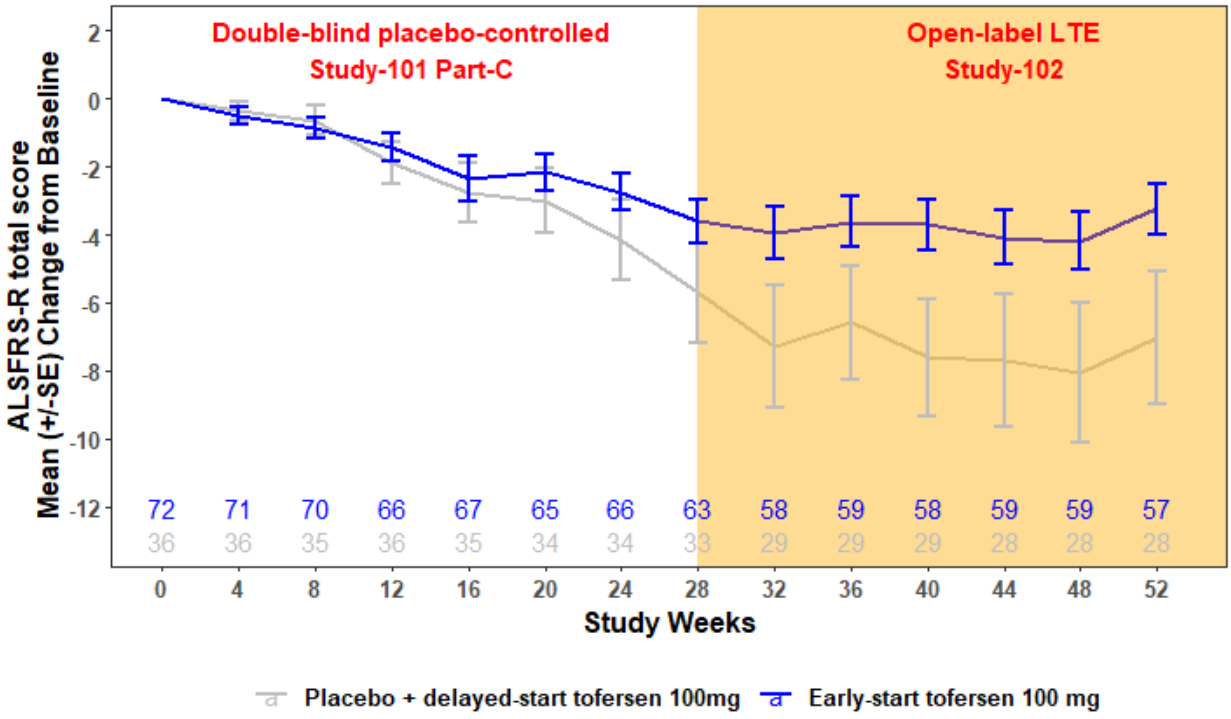
3.1.2.3.2 Evaluation of the long-term treatment effects

The total CSF SOD1 change from baseline profile following tofersen treatment showed a clear reduction of total SOD1 protein starting at Week 8. The total CSF SOD1 reached an approximate maximal reduction in the tofersen treatment group starting at Week 16. Thus, any potential treatment effect of tofersen on clinical function, a downstream effect of tofersen's biological effects on SOD1 protein, is likely to be delayed. Therefore, it is hypothesized that longer treatment duration would offer a greater opportunity for tofersen to demonstrate a clinical benefit.

To explore the long-term treatment effect of tofersen, the Clinical Pharmacology review team conducted an independent, post hoc analysis to compare the ALSFRS-R changes at week 52 from the integrated data from Study 101C and long-term extension Study 102 within the ITT population. When evaluating the long-term effect of tofersen, participants in the early-start group (those who initiated tofersen in Study 101C) were compared with participants in the placebo/delayed start group (i.e., those who had the opportunity to receive tofersen in Study 102 after 28 weeks on placebo) (Figure 17). Based on the observed data of ALSFRS-R total score, the early-start tofersen group has shown numerically less decline in ALSFRS-R total score as compared to delayed-start group, which is consistent from Week 28 to Week 52. If one assumes that tofersen acts like a placebo, starting treatment 28 weeks earlier (or later) would not be anticipated to change the course of the disease or impact disease progression. In that case, the ALSFRS-R curves between the early-start group and the placebo/delayed-start group should overlap, as seen in the first 8 weeks. Nevertheless, the consistent separation on ALSFRS-R between the two groups from Week 8 and onwards appears to further support the potential treatment effect of tofersen. We acknowledge the limitation that after Week 28, the trial enters open-label phase and all patients started to receive the same active treatment. However, as noted above, enrolled patients, site staff, and vendors were still blinded by the initial treatment assignment even after entering the open-

label phase so it is unlikely that the initial treatment assignment would significantly affect the ALSFRS-R assessment in the open-label phase.

Figure 17: Longitudinal Change In Observed Mean (±SE) ALSFRS-R Over 52 Weeks in Study Completers Of ITT Population Of Study 101 Part-C



Source: Clinical Pharmacology Reviewer's Analysis

It is also important to note that a reduction in NfL is seen in patients who switch from placebo to active treatment in the OLE, with a magnitude of effect similar to that seen in Study 101C (Figure 4). Although it is important to note that it is a smaller number of patients due to the 2:1 randomization as well as drop-out going into the OLE, but a mean 44% reduction in NfL is seen in patients after switching to active treatment. These patients also subsequently had a slowing of decline in the ALSFRS-R Figure 17. This provides additional support that a reduction in NfL in these patients may be able to predict future clinical benefit.

3.1.2.4 Summary Comments on NfL as a Reasonably Likely Surrogate Endpoint

As part of the 21 CFR 314.510 Subpart H regulations, FDA ,may exercise its broad scientific judgment in applying the evidentiary approval standards to drugs for life-threatening and severely debilitating diseases, especially where there is no satisfactory alternative therapy. In addition, the accelerated approval regulations build upon this recognition by acknowledging that reliance on a surrogate endpoint "almost always introduces some uncertainty into the risk/benefit assessment, because clinical benefit is not measured directly and the quantitative relation of the effect on the surrogate to the clinical effect is

rarely known." Together, these regulations recognize the importance of facilitating the development of, and access to, safe and effective treatment options for life-threatening and severely debilitating diseases with unmet medical needs. This approach has been reinforced by FDA's interactions with patients and their caregivers who describe their willingness to accept less certainty about effectiveness in return for earlier access to much needed medicines.

There are many types of biomarkers that can have different clinical and regulatory use. In this setting of considering reliance on a biomarker to support accelerated approval, the biomarker is being used as a reasonably likely surrogate endpoint, defined as "an endpoint supported by strong mechanistic and/or epidemiologic rationale such that an effect on the surrogate endpoint is expected to be correlated with an endpoint intended to assess clinical benefit in clinical trials, but without sufficient clinical data to show that it is a validated surrogate endpoint."[40]

Accelerated approval may be granted for a product for a serious or life-threatening disease upon a determination that the product has an effect on a reasonably likely surrogate endpoint that is not itself a direct measure of the clinical benefit of interest but is instead reasonably likely to predict that clinical benefit. When granting accelerated approval, it is expected that there will be empirical evidence that the observed change in the biomarker after administration of the drug is likely to predict clinical benefit. This empirical evidence is disease specific, depends on the natural history of the disease, and the adequacy of the evidence to support use of the surrogate endpoint is based on the biologic plausibility of the relationship between the disease and the biomarker, and the magnitude of observed change in the biomarker that supports the relationship.

The evaluation of whether an effect of tofersen on NfL in SOD1-ALS is reasonably likely to predict clinical benefit is multi-disciplinary and involves important considerations related to the understanding of the disease pathology and the mechanism of action of tofersen, as well as statistical analyses to evaluate the prognostic value of plasma NfL levels in ALS and the relationship between drug effects on NfL and drug effects on clinical endpoints. Below provides some summary comments on the evaluation of plasma NfL as a reasonably likely surrogate endpoint, including perspectives from both the clinical pharmacology review team and the statistical review team.

Clinical Pharmacology Conclusions

The review focused on evaluating plasma NfL as a reasonably likely surrogate endpoint for SOD1-ALS to provide support for accelerated approval. This was supported by 1) The understanding of the pathophysiology of SOD1-ALS and the pharmacology of tofersen which provides mechanistic support that plasma NfL is a biomarker that is reasonably likely to predict clinical function. 2) The prognostic value of plasma NfL in ALS that was demonstrated by leveraging data from literature and Study 101C. 3) The relationship between tofersen-driven NfL reduction and changes in clinical decline. This relationship was evaluated using longitudinal changes in NfL and ALSFRS-R total score, correlation analysis and causal inference analysis. We note that the effect of NfL reduction on the clinical endpoints from the causal

[40] https://www.ncbi.nlm.nih.gov/books/NBK453485/

inference analysis should be interpreted under the context of the model assumptions. Nevertheless, these analyses supported the trend between tofersen-driven NfL reduction and reduction in clinical decline, and to ensure no apparent deviation from some assumptions. Overall, all above-mentioned analyses collectively support plasma NfL as a biomarker that reasonably likely predicts the clinical outcome change.

Biostatistical Comments

The statistical review focuses on general statistical issues with evaluating candidate surrogate endpoints, and on the ability of analyses of NfL and clinical endpoint data from Study 101C (and its OLE, Study 102) to support NfL as a reasonably likely surrogate endpoint.

The Applicant reported extensive post hoc, exploratory analyses. Post-hoc selection of timepoints, endpoints, and covariates risks introduces bias in the evaluation due to the data-driven nature of the analyses. Furthermore, it is challenging to assess whether a drug effect on a biomarker reasonably likely predicts a drug effect on a clinical outcome from a study that did not provide evidence of an effect on the clinical outcome.

The Applicant has provided support for the prognostic value of NfL in ALS, i.e., providing data from the literature showing that higher NfL levels correlate with higher risk of unfavorable outcomes. In addition, there is some correlation between changes in NfL and changes in ALSFRS-R in Study 101C. However, the magnitude of the correlation is small and may be influenced by potentially data-driven analysis choices such as endpoint selection, the scale for NfL, and selection of covariates in adjusted models. Furthermore, it is important to emphasize that correlation between a biomarker and clinical endpoint is necessary but not sufficient to support that a drug effect on the biomarker will reliably predict a drug effect on the clinical endpoint. Notably, even the presence of strong correlation between the biomarker and the clinical endpoint within both the placebo and drug arms of a clinical trial may not be sufficient. It is therefore often important to consider additional analyses and data sources that go beyond such correlation. See further discussion in Fleming, Thomas R., and John H. Powers. "Biomarkers and surrogate endpoints in clinical trials."

The Applicant has also conducted causal inference analyses to explore the relationship between tofersen effects on NfL and ALSFRS-R. Although the model may inform the relationship between changes in NfL and changes in clinical outcomes, it cannot conclusively establish the causal relationship between tofersen effects on NfL and ALSFRS-R. First, the model was developed after the unblinding of the study data and was likely driven by the observed data. Second, unlike an analysis based on a randomized comparison, the validity of the results depends on the form of the model, the variables included in the model, and the specific data used to fit the model. Third, the estimation of the uncertainty of the results depends on assumptions about the statistical error terms and missing data, which may not hold for the present model. For example:

- The analysis assumes that a natural NfL progression model based on only the placebo arm with 8.5% missing data is correct, with no error added to prediction for the active arm's counterfactual placebo NfL within the causal model. This is despite the exponential transformation back to the original NfL scale which might be less reliable and highly variable,

especially for high baseline NfL. Therefore, the variability of the estimated causal effect is underestimated.

- The variances in the mITT and non-mITT population are assumed equal. However, the residual unexplained variance is higher for the mITT (fast progressors) than the non-mITT population as expected at the design stage (e.g., residual variance estimates are 26.6 and 4.7 for mITT and non-mITT populations, respectively, for placebo in the subgroup analyses).
- The analysis may be confounded by missing data or death which is assumed missing completely at random (i.e., not dependent on any variables or missing outcomes), and there were 12.5% of tofersen subjects and 8% of placebo subjects with missing values (including 1 death on tofersen).

It is also unclear whether the Applicant conducted model validation to assess how well their model(s) could predict the treatment effect on clinical outcomes. Model validation involves validation from independent data not used to build the model.

One alternative approach discussed in the statistical literature for assessment of surrogacy is to explore the extent to which the estimated treatment difference for the clinical endpoint, ALSFRS-R, may be explained by the treatment difference for the biomarker, NfL. Of note, in this application, such an approach may be greatly limited by the fact that the trial did not provide evidence of an effect on the clinical endpoint.

The Applicant also submitted details of an extension of their causal model to the survival analyses on February 14, 2023. The review team has not fully reviewed it, but it appears to have similar limitations as the causal model for ALSFRS-R and other functional endpoints, along with potential additional uncertainties due to the small numbers of survival endpoint events that have occurred.

3.1.3 Efficacy Conclusions

SOD1-ALS is a very rare disease with a very limited population available for clinical studies. Tofersen is a targeted therapy that targets SOD1 mRNA, decreasing transcription of SOD1 protein, the toxic form of which is implicated in the pathophysiology of the disease. Study101C, a placebo-controlled, double-blind study failed to win on the prespecified primary analysis; however, the study showed some non-significant trends on clinical outcomes that favored tofersen, and convincing reductions in biomarkers that are implicated in the pathophysiology of SOD-1 ALS. Reductions in CSF SOD1 protein (nominal p-value < 0.0001) provides evidence of target engagement. Tofersen treatment also led to 55% reduction (nominal p-value < 0.0001) in plasma NfL, which is likely evidence of a downstream decrease in neuronal damage of motor neurons in SOD1-ALS patients. Based on the pathophysiology of SOD1-ALS, it follows that such a reduction in neuronal damage, as evidenced by reduction in NfL, could be expected to lead to slower clinical functional decline.

When considering the data from Study 101C and the OLE combined, there appears to be clinical trends towards separation over time between the treatment groups on the primary and secondary endpoints, which persist through Week 52, although there are limitations with these analyses as discussed above.

There are a number of different approaches that can be taken to analyze this data, based on different statistical assumptions, covariates, and populations. In this document, the Agency has emphasized analyses based on the analytical methods prespecified prior to unblinding of the data from Study 101C. In the Applicant's briefing document, they have emphasized analytical methods based on learning from analysis of the unblinded data of Study 101C, as well as from scientific knowledge gained external to the study. Regardless of the statistical approaches used, there appear to be generally consistent trends favoring tofersen across the different statistical methods.

To support tofersen for accelerated approval, plasma NfL has been evaluated as a reasonably likely surrogate endpoint for SOD1-ALS based on the following aspects:

- There is mechanistic evidence that tofersen reduces SOD1 protein, the intended target of the drug and known contributor to the pathophysiology of neuronal degeneration in patients with SOD1-ALS and also reduces NfL, a biomarker of neurodegeneration that is known to be substantially elevated in patients with ALS and predictive of disease progression.
- Evidence from the literature as well as clinical programs have demonstrated the prognostic value of plasma NfL in predicting disease progression and survival in ALS.
- Additionally, there is an observed correlation between reduction in NfL and a slowing of decline on clinical outcomes such as the ALSFRS-R, and a causal inference model which, despite statistical limitations, appear to support the use of NfL as a biomarker reasonably likely to predict clinical benefit in patients with SOD1-ALS.

Despite the notable limitations of a failed study and the many post hoc exploratory analyses that were conducted after Study 101C, the Division considers that the data may suggest a treatment effect of tofersen in SOD1-ALS. This a very rare and devastating disease; therefore, it is of utmost importance that we give full consideration to all of the available data. The following factors could have reduced the ability of the study to detect a drug effect, if there is one, with tofersen.

- Decline in both the placebo and treatment groups was much less than expected, leading to the study being greatly underpowered.
- As noted above, the mITT population was enriched for "fast-progressors" based on pre-randomization slope and genetic mutation. However, the observed data on post-randomization slope/disease progression among placebo patients suggested that the heterogeneity of the disease progression rate was not well-controlled in the mITT population when relying on mutation type and pre-randomization slope.
- The imbalance of NfL baseline in the mITT population may have predicted faster progression in the tofersen arm, which would have placed a disadvantage for the tofersen group compared to the placebo and decreased the ability to detect a treatment effect if it is there.
- Additionally, a 28-week treatment duration may not have been sufficient time to observe a treatment benefit, particularly given that tofersen-driven NfL reductions do not appear to reach a maximum effect as until Week 16.

Given these considerations, we seek the input from the advisory committee on the strength of the efficacy data to support a treatment effect of tofersen in SOD1 ALS in two scenarios:

The first scenario would invoke the accelerated approval pathway which allows approval of a drug based on an effect on a surrogate endpoint that is found to be reasonably likely to predict clinical benefit. The surrogate endpoint serves as an indirect measure of clinical benefit, and under this pathway, confirmation of the clinical benefit is required, which usually comes from an adequate and well-controlled study. In this situation, we are considering whether the available evidence supports that the reduction in NfL observed in tofersen-treated patients with amyotrophic lateral sclerosis (ALS) secondary to a mutation in SOD1 (SOD1-ALS) is reasonably likely to predict clinical benefit for these patients.

The second scenario considers whether the available data is strong enough, in this rare disease population, that clinical trends and nominally significant benefits in the OLE at week 52 may be sufficient, combined with confirmatory evidence of reduction in SOD1 and NfL, to establish a treatment benefit of tofersen in SOD1-ALS to support full approval of the drug.

In this setting of a very rare, life-threatening disease with significant unmet need, it is appropriate to exercise regulatory flexibility in applying the statutory standards for establishing effectiveness. For example, FDA's regulation at 21 CFR 312.80 notes, "while the statutory standards of safety and effectiveness apply to all drugs, the many kinds of drugs that are subject to them, and the wide range of uses for those drugs, demand flexibility in applying the standards. The Food and Drug Administration (FDA) has determined that it is appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness." This approach is reiterated in FDA's guidance for industry, ALS: Developing Drugs for Treatment (September 2019), which states: "The statutory standards for effectiveness apply to drugs for ALS just as the standards apply for all other drugs. However, FDA has long stressed the appropriateness of exercising regulatory flexibility in applying the statutory standards to drugs for serious diseases with unmet medical needs, while preserving appropriate assurance of safety and effectiveness."

SOD1-ALS is clearly such a severely debilitating and life-threatening disease with substantial unmet need. FDA also recognizes the importance of considering patient tolerance for risk and the nature of the condition in the context of statutory requirements for safety and efficacy.

3.2 Safety Issues

As outlined below, the main safety signal in the tofersen development program is the potential risk of serious neurologic adverse events that appear to be associated with the intrathecal route of administration of tofersen (i.e., myelitis, radiculitis, aseptic meningitis, papilledema, and elevated intracranial pressure). Given the serious and life-threatening nature of ALS, these risks do not appear to preclude approval and risk can be adequately mitigated through a description in labeling.

3.2.1 Sources of Data for Safety

Safety was assessed by evaluating the results from the randomized, placebo-controlled study, Study 101C, and the open-label extension Study 102 in adult subjects with SOD1-ALS. See Table 1 above.

3.2.2 Safety Summary

Total exposure in the double-blind controlled Study 101C for patients randomized to tofersen (N = 72) was a median of 28.1 weeks. The median exposure in the combined Study 101 and Study 102 integrated database (N = 147) was 119.4 weeks. A total of 116 patients received tofersen for > 48 weeks. Given the rarity of SOD1-ALS, the safety database is considered adequate.

In the double-blind, placebo-controlled database from Study 101C, the most commonly observed adverse events (AEs) (occurring in ≥ 10% of tofersen treated patients and >5% higher frequency than placebo) associated with the use of tofersen were pain, myalgia, arthralgia, fatigue, and CSF white blood cell increased. The most common AEs remained similar in the open-label Study 102 as well.

The proportion of subjects experiencing serious adverse events (SAEs) was 18% in the tofersen group and 14% in the placebo group. SAEs that occurred in more than one subject in the pooled studies 101C and 102 include respiratory disorders (SOC) (19%), pneumonia aspiration (7%), dysphagia (5%), pneumonia (2%), intracranial pressure increased (2%), fall (2%), COVID-19 (1%), myelitis (1%), septic shock (1%), back pain (1%), ALS (1%), nephrolithiasis (1%), and faecaloma (1%). Most of these SAEs are consistent with the natural history of ALS, although myelitis and intracranial pressure increased are discussed in more detail below.

There was one death in the tofersen arm in the 28-week placebo-controlled study. This patient died from congestive heart failure, which was likely related to the subject's medical history of coronary artery disease, two prior myocardial infarctions, hypertension, and type 2 diabetes mellitus.

Permanent dose discontinuation due to AEs occurred in 6% of the tofersen group and 0% of the placebo group. The adverse events that caused these 4 subjects to stop taking tofersen in the placebo-controlled study were cardiac failure congestive, myelitis, meningitis chemical, and pulmonary embolism, respectively. The adverse events that caused the most subjects to stop taking tofersen across the pooled clinical studies were respiratory failure (8%), respiratory arrest (1%), and ALS (1%).

There were no clinically significant differences in the proportions of subjects with AEs or SAEs as a function of age or sex subgroup for Study 101C.

There were also serious neurologic events that occurred in patients treated with tofersen that did not occur in patients receiving placebo and warrant further discussion. These events include myelitis and radiculitis, aseptic and chemical meningitis, and papilledema and increased intracranial pressure, and appear to be associated with the intrathecal route of administration of tofersen.

An SAE of myelitis occurred in four subjects (3%) in combined studies 101C and 102 who received tofersen and radiculitis occurred in two subjects (1%). No subjects receiving placebo in study 101C

experienced these SAEs. The SAEs of myelitis led to tofersen discontinuation in two patients, one of whom was ultimately diagnosed with neurosarcoid transverse myelitis, and another with an inflammatory myelopathy resulting in paraplegia and T10 sensory loss who recovered within 2 months after tofersen discontinuation. The other two patients with myelitis were asymptomatic and continued on treatment. An additional patient in the expanded access program also reported symptomatic myelitis leading to treatment discontinuation. Both patients who reported SAEs of radiculopathy continued on tofersen and had resolution of symptoms.

Two subjects who received tofersen had SAEs of either aseptic meningitis (one subject [1%] in Study 102) or chemical meningitis (one subject [1%] in Study 101C). No subjects receiving placebo in study 101C experienced these SAEs. Aseptic meningitis and chemical meningitis have been reported with other intrathecally (IT) administered treatments, including reports of aseptic meningitis with IT administration of another ASO. Aseptic meningitis may be caused by hypersensitivity reactions or direct meningeal irritation, and often is diagnosed based on CSF findings of pleocytosis and elevated CSF protein. There were reports of nonserious AEs of elevated CSF WBC and CSF pleocytosis in Study 101C; however, the single reported SAE of chemical meningitis in Study 101C did lead to treatment discontinuation, with complete resolution 2 weeks after symptom onset. An SAE of aseptic meningitis was also reported in the open-label Study 102 in a single patient who also reported myelitis and increased intracranial pressure (see below), which resolved with treatment and did not lead to permanent discontinuation of tofersen.

In the pooled studies 101 (Parts A, B, and C) and Study 102 there were 4 of 139 (3%) subjects with an SAE of papilledema or intracranial pressure increased, compared to 0% in the placebo group of Study 101C. None of these led to permanent discontinuation of tofersen. One of these patients also had SAEs of aseptic meningitis and asymptomatic myelitis, and another one had aseptic meningitis. There were also additional nonserious reports of papilledema and increased intracranial pressure increased without discontinuation.

3.2.3 Safety Conclusion

In summary, the most commonly observed AEs associated with the use of tofersen were pain, myalgia, arthralgia, fatigue, and CSF white blood cell increased. Many of the reported SAEs were related to underlying disease progression rather than drug-related and there were no fatal adverse events.

There is a risk for serious neurologic events that were reported in patients receiving tofersen but not in patients receiving placebo. The majority of these events, including the SAEs of radiculitis, papilledema, increased intracranial pressure, and aseptic meningitis may be related to the route of administration rather than specific to the drug itself. We note reports of similar findings with other ASOs that are administered intrathecally. The majority of these events resolved and did not lead to permanent discontinuation of therapy. The risk of myelitis has been described in 5 patients receiving tofersen (4 in the clinical trials and 1 patient in expanded access program), three of whom discontinued treatment secondary to the adverse event. Patients and providers would need to be aware of this potential serious risk.

3.3 Risk Mitigation

The risks of myelitis, radiculitis, drug-induced aseptic meningitis, papilledema, and elevated intracranial pressure appear related to drug and/or route of administration. These risks are acceptable to this patient population, and would not preclude approval. If tofersen is approved, these risks should be described in the Warnings and Precautions section of the prescribing information.

Given the severity of ALS relative to the observed risks in the tofersen studies, a Risk Evaluation and Mitigation Strategy (REMS) is not recommended at this time.

4 References

Refer to footnotes throughout document.

5 Appendix

5.1 Causal inference Analysis

The objective of the causal inference analysis was to evaluate the relationship between the tofersen-driven reduction in plasma NfL at Day 113 (Week 16) and changes in clinical outcome measures (ALSFRS-R total score, percent predicted SVC, HHD megascore, ALSDAQ-5 total score, and EQ-5D-5L utility score) at Day 197 (Week 28).

Data from ITT population (N=108) of Study 233AS101 Part C was used. The model partitioned the effect of tofersen on the change from baseline in clinical endpoint at Week 28 into three components: (i) natural disease progression, (ii) drug effect via NfL pathway, and (iii) drug effect via non-NfL pathway. Figure 18 summaries the key equations used in the causal inference models. Briefly, equation 2.1 model log-transformed NfL at Day 113 as a linear function of log-transformed baseline NfL and age in the placebo arm. Equation 2.2. model the change from baseline in clinical endpoint in the placebo arm as a linear function of a baseline NfL, change in NfL, and other baseline covariates for clinical function. The third equation 2.3 model the change from baseline in clinical endpoint in the active arm as a linear function of natural disease progression and tofersen effect via non-NfL and non-biomarker pathway. These regression equations were solved using maximum likelihood approach.

Figure 18: List of Equations utilized in the Causal Inference Modeling

$$\log(z_{0it_1}) = \alpha_{0,z} + \beta_{0,z}\log(z_{0it_0}) + \sum_{j=1}^{\cdot}\beta_{0,z}^{(j)}v_{0ij} + \epsilon_{0it_1,z} \quad (2.1)$$

$$\Delta y_{0it_2}|\Delta z_{0it_1} = \alpha_{0,y} + \beta_{0,y}z_{0it_0} + \gamma_{0,y}\Delta z_{0it_1} + \sum_{j=1}^{m_2}\beta_{0,y}^{(j)}w_{0ij} + \epsilon_{0it_2,y} \quad (2.2)$$

$$\begin{aligned}\Delta y_{1it_2}|\Delta z_{1it_1} = {} & \alpha_{0,y} + \beta_{0,y}z_{1it_0} + \gamma_{0,y}\left[e^{\alpha_{0,z}+\beta_{0,z}\log(z_{1it_0})+\sum_{j=1}^{m_1}\beta_{0,z}^{(j)}v_{1ij}} - z_{1it_0}\right] + \sum_{j=1}^{m_2}\beta_{0,y}^{(j)}w_{1ij} + \\ & \lambda_0 + \sum_{j=1}^{m_3}\lambda_j u_{1ij} + \\ & (\gamma_1 + \gamma_2 z_{1it_0,\text{std}})\left\{\Delta z_{1it_1} - \left[e^{\alpha_{0,z}+\beta_{0,z}\log(z_{1it_0})+\sum_{j=1}^{m_1}\beta_{0,z}^{(j)}v_{1ij}} - z_{1it_0}\right]\right\} + \\ & \epsilon_{1it_2,y} \end{aligned} \quad (2.3)$$

z_{0it0}: Baseline NfL for subject *i* in the placebo arm.
z_{1it0}: Baseline NfL level for subject *i* in the active arm.
z_{0it1}: NfL level for subject *i* in the placebo arm at Week 16.
z_{1it1}: NfL level for subject *i* in the active arm at Week 16.
Δz_{0it1}: Change from baseline in NfL at Week 16 for subject *i* in the placebo arm.
Δz_{1it1}: Change from baseline in NfL at Week 16 for subject *i* in the active arm.
$z_{1it0,std}$: Standardized baseline NfL level for subject *i* in the active arm.

y_{0it2}: Functional endpoint at Week 28 for subject *i* in the placebo arm.
y_{1it2}: Functional endpoint at Week 28 for subject *i* in the active arm.
Δy_{0it2}: Change from baseline in functional endpoint at Week 28 for subject *i* in the placebo arm.
Δy_{1it2}: Change from baseline in functional endpoint at Week 28 for subject *i* in the active arm.
v_{0i}: vector of fixed standardized covariates for NfL for subject *i* in the placebo arm.
v_{1i}: vector of fixed standardized covariates for NfL for subject *i* in the active arm.
w_{0i}: vector of fixed standardized covariates for clinical function for subject *i* in the placebo arm.
w_{1i}: vector of fixed standardized covariates for clinical function for subject *i* in the active arm.
u_{1i}: vector of fixed standardized covariates explaining the drug effect on the non-biomarker pathway for subject *i* in the active arm.

Source: Section 2.7.2 Summary of Clinical pharmacology studies Appendix 1, page 5

5.2 Timeline of Important Study and Documentation Dates

Table 1: Important Study Dates	
Study Number	233AS101-Part C
Date of First Treatment	3/27/2019
End of Study Date	7/16/2021
Report	4/12/2022

(Source: Stats Reviewer's own analysis)

Table 2: Important Study Documentation Version/Dates				
Study Number: 233AS101 Part C	Version	DATE on document	IND 124264	Stamp Date
Protocol	1	9/24/2015	SN 0	10/28/2015
	2	10/10/2016	SN 6	10/26/2016
	3	11/17/2016	SN 11	3/7/2017
	4	11/17/2017	SN 16	12/13/2017
Part C	5	12/20/2018	SN 30	1/15/2019
Part C	6	9/19/2019	SN 47	10/18/2019
Part C	7	9/30/2019	SN 50	10/18/2019
Part C	8	6/15/2021	SN 144	6/28/2021

SAP				
	1	6/9/2021 -> 6/9/2019	SN 50	10/18/2019
	2	8/14/2021	SN 150	8/15/2021

(Source: Biostats Reviewer's own analysis using EDR submissions.)

5.3 Additional Statistical Concerns

Issues with Sample Size Planning

In the Study Report, the sample size justification was based on an assumed mean slope of decline of -3.83 per month for the placebo participants (i.e., 24.7-point decline over 28 weeks) and -0.74 per month for the tofersen 100 mg participants (approximately a 4.8 decline over 28 weeks), with a pooled SD of 3.166. Furthermore, the survival at Week 28 was assumed to be 82% in the placebo control and 90% in the tofersen 100 mg group. Under the above assumptions, with N = 60 participants in the mITT population and a two-sided significance level of 0.05, the Joint Rank Test gave 84% power.

The study actually had 60 patients in the mITT population (as planned) and the survival rates were better than expected. One reason the study failed may have been that decline in both the placebo and treatment groups was much less than expected. The actual observed decline over 28 weeks was approximately an 8.1 decline in the placebo arm and a 7-point decline in the tofersen arm, a mean difference of about 1 point instead of the difference of 20 that was assumed. The survival rate in both arms combined was 59/60 (one death in the tofersen arm). Assuming these observed rates of decline and a common survival rate of 59/60 and leaving the other assumptions unchanged, a future study would need 12,000 participants to achieve 80% power to detect a mean difference of 1.1 points decline in ALSFRS-R. However, there is some uncertainty about the effect and it may be larger than 1.1. Also, the effect may be larger with longer follow-up. If the effect is larger than 1.1., a smaller sample size may be adequate.

Issues with Method of Imputation of Missing Data

For the prespecified statistical methods (i.e., joint rank or ANCOVA) for the primary endpoint, multiple imputation using MCMC under the normality assumption was used to handle missing data or incompleters. The statistical analysis plan did not specify an alternative method for handling missing data in case that the normality assumption did not hold. The Applicant did not check the normality assumption.