ORIGINAL ARTICLE

Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS

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ABSTRACT

BACKGROUND

The intrathecally administered antisense oligonucleotide tofersen reduces synthesis of the superoxide dismutase 1 (SOD1) protein and is being studied in patients with amyotrophic lateral sclerosis (ALS) associated with mutations in *SOD1* (*SOD1* ALS).

METHODS

In this phase 3 trial, we randomly assigned adults with *SOD1* ALS in a 2:1 ratio to receive eight doses of tofersen (100 mg) or placebo over a period of 24 weeks. The primary end point was the change from baseline to week 28 in the total score on the ALS Functional Rating Scale–Revised (ALSFRS-R; range, 0 to 48, with higher scores indicating better function) among participants predicted to have faster-progressing disease. Secondary end points included changes in the total concentration of SOD1 protein in cerebrospinal fluid (CSF), in the concentration of neurofilament light chains in plasma, in slow vital capacity, and in handheld dynamometry in 16 muscles. A combined analysis of the randomized component of the trial and its open-label extension at 52 weeks compared the results in participants who started tofersen at trial entry (early-start cohort) with those in participants who switched from placebo to the drug at week 28 (delayed-start cohort).

RESULTS

A total of 72 participants received tofersen (39 predicted to have faster progression), and 36 received placebo (21 predicted to have faster progression). Tofersen led to greater reductions in concentrations of SOD1 in CSF and of neurofilament light chains in plasma than placebo. In the faster-progression subgroup (primary analysis), the change to week 28 in the ALSFRS-R score was −6.98 with tofersen and −8.14 with placebo (difference, 1.2 points; 95% confidence interval [CI], −3.2 to 5.5; P=0.97). Results for secondary clinical end points did not differ significantly between the two groups. A total of 95 participants (88%) entered the open-label extension. At 52 weeks, the change in the ALSFRS-R score was −6.0 in the early-start cohort and −9.5 in the delayed-start cohort (difference, 3.5 points; 95% CI, 0.4 to 6.7); non–multiplicity-adjusted differences favoring early-start tofersen were seen for other end points. Lumbar puncture–related adverse events were common. Neurologic serious adverse events occurred in 7% of tofersen recipients.

CONCLUSIONS

In persons with *SOD1* ALS, tofersen reduced concentrations of SOD1 in CSF and of neurofilament light chains in plasma over 28 weeks but did not improve clinical end points and was associated with adverse events. The potential effects of earlier as compared with delayed initiation of tofersen are being further evaluated in the extension phase. (Funded by Biogen; VALOR and OLE ClinicalTrials.gov numbers, NCT02623699 and NCT03070119; EudraCT numbers, 2015-004098-33 and 2016-003225-41.)

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A Quick Take is available at NEJM.org

Approximately 2% of cases of amyotrophic lateral sclerosis (ALS) are associated with mutations in the gene encoding superoxide dismutase 1 (*SOD1*).[1,2] More than 200 ALS-associated *SOD1* mutations have been described and are associated with variable rates of progression.[3-7] Neuronal degeneration in this disorder is considered to be caused by toxic gain of function of the mutant SOD1 protein.[1,8-12] Tofersen is an intrathecally administered antisense oligonucleotide designed to reduce the synthesis of SOD1 protein by inducing RNase H–mediated degradation of *SOD1* messenger RNA.[10,13-15] We conducted a 28-week, phase 3, randomized trial of the efficacy and safety of tofersen in adults with *SOD1* ALS. This is part C (VALOR) of a three-part trial, the first two parts of which were dose-escalation trials conducted to assess the dose of tofersen to be used in part C (see the protocol, available with the full text of this article at NEJM.org).[15] Participants who were enrolled in parts A and B were not enrolled in part C. After completion of this trial, participants had the opportunity to enroll in an ongoing open-label extension.

METHODS

TRIAL OVERSIGHT

The trials were conducted in accordance with the Good Clinical Practice guidelines of the International Council for Harmonisation and the ethical principles outlined in the Declaration of Helsinki. The protocols of VALOR and its open-label extension were approved by relevant ethics committees. An independent data monitoring committee reviewed safety data. Written informed consent for both the randomized phase and open-label extension was provided by participants or their legal representatives.

The sponsor, Biogen, and the authors designed these trials. Biogen provided tofersen and placebo, oversaw the trial, performed the statistical analyses, and paid for medical writing assistance. Biogen and the authors analyzed the data. The first draft of the manuscript was written by the first author and an author employed by Biogen. The sponsor reviewed the manuscript but could not delay or prevent publication of the results. The authors reviewed and approved revisions of the manuscript and vouch for the accuracy and completeness of the data, the fidelity of the trials to the protocols, and the accuracy of the reporting of adverse events. There were confidentiality agreements between the authors and Biogen.

TRIAL DESIGN

The phase 3, double-blind, randomized, placebo-controlled VALOR component of the trial was conducted from March 2019 through July 2021.[16] Participants were enrolled at 32 sites in 10 countries (see Section S1 in the Supplementary Appendix, available at NEJM.org). The trial included a 4-week screening period, a 24-week treatment period, and a follow-up period of 4 to 8 weeks followed by an ongoing extension phase.

Participants were randomly assigned in a 2:1 ratio to receive an intrathecal bolus injection through a lumbar puncture of a 15-ml solution of tofersen (100 mg) or an equivalent volume of placebo (artificial cerebrospinal fluid [CSF]) administered over a period of 24 weeks, as three doses once every 2 weeks, followed by five doses once every 4 weeks (Fig. S1 in the Supplementary Appendix). Randomization was stratified according to the use or nonuse of edaravone, riluzole, or both at baseline and according to whether participants met prognostic criteria for faster disease progression that were based on *SOD1* mutation type and the estimated slope of the score on the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R), calculated from the time of symptom onset until screening ("prerandomization ALSFRS-R slope"). Owing to the potential for nonlinear progression on the ALSFRS-R score and for intra-mutation variability confounding the prognostic value of these measures, as well as literature supporting the use of neurofilament light chains as a prognostic marker of disease progression,[16-24] analyses in subgroups that were defined according to baseline concentrations of neurofilament light chains in plasma (above vs. below the median concentration for the trial population) were prespecified before VALOR results were available (see Section S2 in the Supplementary Appendix).

After completion of VALOR, participants were given the option to participate in an open-label extension for up to 236 weeks, while remaining unaware of their trial-group assignment in VALOR. The combined analysis at week 52 of VALOR and its open-label extension was prespecified and was intended to enable comparison of early-start

and delayed-start tofersen in the full intention-to-treat population. The extension phase is ongoing, and analysis is planned when all participants have completed at least 3.5 years of follow-up, which has not been reached.

PARTICIPANTS

We enrolled adults with weakness attributable to ALS and a confirmed *SOD1* mutation. The primary analysis population was the subgroup of participants who met the trial-defined prognostic criteria for faster-progressing disease (see Section S2 in the Supplementary Appendix) and is called the "faster-progression subgroup." Also undergoing randomization was a subgroup of participants who did not meet these enrichment criteria and were predicted to have slower progression of disease, the slower-progression subgroup. These persons were not included in the primary end-point analysis but had the opportunity to enroll in the open-label extension to receive tofersen. In the evaluation of combined data from VALOR and its open-label extension, participants who initiated tofersen in VALOR are referred to as the "early-start cohort," regardless of whether they were predicted to have faster-progressing or slower-progressing disease in the randomized part of the trial. Those who received placebo in VALOR and had the opportunity to cross over to tofersen in the open-label extension approximately 28 weeks later are referred to as the "delayed-start cohort."

END POINTS

The primary efficacy end point in VALOR was the change from baseline to week 28 in the ALSFRS-R total score in the faster-progression subgroup. The ALSFRS-R consists of 12 items across four subdomains of function (bulbar, fine motor, gross motor, and breathing), with total scores ranging from 0 to 48 and higher scores indicating better function. Prespecified secondary end points included the change from baseline in the total concentration of SOD1 protein in CSF, the concentration of neurofilament light chains in plasma, the percentage of the predicted slow vital capacity (volumes were standardized to the percentage of the predicted normal value on the basis of age, sex, and height), the handheld dynamometry megascore (average of z-scores across 16 muscle groups in the arms and legs, with higher values indicating greater strength), the time to death or permanent ventilation (≥22 hours of mechanical ventilation per day for ≥21 consecutive days), the time to death, and safety. Prespecified exploratory end points included participant-reported outcome measures such as the five-item Amyotrophic Lateral Sclerosis Assessment Questionnaire, fatigue (Fatigue Severity Scale), and quality of life (EuroQol Group 5-Dimension questionnaire). The same end points were assessed as part of the combined analyses of VALOR and the open-label extension.

STATISTICAL ANALYSIS

We calculated that a sample size of 60 participants (2:1 randomization ratio) in the faster-progression primary analysis subgroup would provide 84% power to detect a between-group difference on the basis of the joint rank test (described below), assuming a change in the ALSFRS-R score from baseline to week 28 of −4.8 in the tofersen group and −24.7 in the placebo group, with a standard deviation of 20.39 and survival of 90% in the tofersen group and 82% in the placebo group, at a two-sided alpha level of 0.05. All primary and secondary end points for the 28-week randomized part of the trial were formally tested in the faster-progression subgroup. In the slower-progression subgroup, only the total SOD1 concentration in CSF was powered to test for statistical significance and was the primary end point in this population (Table S3). The joint rank test was used for statistical inference in the analysis of the change in the ALSFRS-R score. This accounts for both functional decline and survival and allows for a statistical test of the treatment effect while accounting for truncation of data owing to deaths. The joint rank score was calculated by comparing the change in each participant's ALSFRS-R score from baseline to week 28 with that of every other participant in the trial, resulting in a score of 1 if the outcome was better than that of the participant being compared, −1 if worse, and 0 if the same. Participants who died were ranked lowest on the basis of their time to death, with progressively lower ranks given to those who died in the shortest period of time after the first dose. The sum of individual scores for each participant (i.e., ranked score) was assessed with the use of analysis of covariance (ANCOVA).

The ANCOVA model for ranked scores on the

ALSFRS-R included trial group as a fixed effect and was adjusted for covariates (baseline disease duration since symptom onset, baseline ALSFRS-R total score, and use of riluzole or edaravone). The estimated between-group difference was obtained from the ANCOVA model for change from baseline in the ALSFRS-R score. Formal statistical testing for the overall population of all randomly assigned participants (irrespective of faster or slower predicted progression) was not specified for VALOR, but estimates are provided from the ANCOVA for change from baseline. Joint rank analysis was performed in conjunction with multiple imputation to account for missing data due to withdrawals not accounted for by death. The multiple-imputation model included trial group, use of riluzole or edaravone, and the baseline ALSFRS-R score. Additional subgroup and exploratory end points and analyses are described in Sections S3 and S4 in the Supplementary Appendix.

If the results for the primary end point differed significantly between the two trial groups, secondary end points for the faster-progression subgroup were tested with the use of a sequential closed testing procedure in order of ranking: the change from baseline (ratio to baseline) to week 28 in the total concentration of SOD1 protein in CSF, the change from baseline (ratio to baseline) to week 28 in the concentration of neurofilament light chains in plasma, the change from baseline to week 28 in the percentage of the predicted slow vital capacity, the change from baseline to week 28 in handheld dynamometry megascore, ventilation assistance–free survival, and overall survival. ANCOVA for change from baseline was used for all continuous end points and in conjunction with multiple imputation for handling missing data for withdrawals. Primary statistical inference for slow vital capacity was by joint rank analysis with the use of multiple imputation. For survival analyses, data for participants who did not meet the end-point definition were censored at the end of the trial or on the date of withdrawal. Only events that were adjudicated by the independent end-point adjudication committee were included. Treatment effects were assessed at a two-sided significance level of 0.05.

The first data cutoff for the combined analysis of data from VALOR and its open-label extension to evaluate the effects of early as compared with delayed initiation of tofersen was performed in July 2021. A second data cutoff of the open-label extension was performed on January 16, 2022, when the last participant who underwent randomization in VALOR had the opportunity for at least 52 weeks of follow-up from the start of VALOR. The combined analyses of these data are presented here. At the time that data from the January 2022 data cutoff were analyzed, the final results from VALOR and the original analysis of VALOR and its open-label extension had been presented at a scientific congress; however, participants, investigators and site staff, and the trial team remained unaware during the extension phase of the original trial-group assignments in VALOR.

Prespecified analyses of the data from VALOR and the data as of the first data cutoff of the open-label extension were performed on the basis of enrichment criteria (fast-progression and slow-progression subgroups) and of categorical subgroups defined by the median concentration of neurofilament light chains in plasma at baseline. Recognizing that adjusting for a continuous variable as a covariate more precisely controls for individual heterogeneity than dichotomizing the population into categorical subgroups, we amended the statistical analysis plan before analysis of the January 2022 data cutoff to incorporate the baseline concentration of neurofilament light chains in plasma as a covariate across analyses (Sections S2 and S4 in the Supplementary Appendix).

The combined analyses of the data as the January 2022 data cutoff are based on the intention-to-treat principle, whereby all participants who underwent randomization in VALOR (108 participants) are included according to their original trial-group assignment, regardless of fast or slow progression, adherence to the trial agent, early termination of the trial, or crossover to the tofersen group. The ANCOVA analyses in conjunction with multiple imputation were conducted identically to the analyses in VALOR. Kaplan–Meier survival analyses included all data up to January 16, 2022, for time to death or permanent ventilation and time to death; between-group comparisons for these end points were based on a log-rank test stratified according to trial group and the median concentration of neurofilament light chains in plasma at baseline (Section S4 and Table S5 in the Supplementary

Appendix). Because there was no plan for adjustment of the widths of confidence intervals for multiple comparisons in the combined analysis, no conclusions can be drawn from these results.

RESULTS

PARTICIPANTS

A total of 108 participants with 42 unique *SOD1* mutations (Table S1) were enrolled in VALOR; 72 were assigned to receive tofersen and 36 to receive placebo. A total of 60 of the 108 participants made up the faster-progression subgroup in which the primary analysis was performed. A total of 95 VALOR participants (88%) were enrolled in the open-label extension (Fig. S3). The amount of missing data for the combined analysis is given below. The clinical characteristics of the participants at baseline were similar in the two trial groups for use of riluzole, edaravone, or both, time from onset of disease symptoms, baseline ALSFRS-R score, and percentage of predicted slow vital capacity. However, baseline concentrations of neurofilament light chains were 15 to 25% higher in participants who received tofersen than in those who received placebo, and the rate of decline in the ALSFRS-R score from screening to day 15 (a period of approximately 42 days) was greater in the participants who received tofersen (Table 1). The mean ALSFRS-R score at baseline was approximately 37 in both groups.

END POINTS

Primary End Point in VALOR

Among the 60 participants in the faster-progression primary analysis subgroup, the change in the ALSFRS-R total score from baseline to week 28 was −6.98 points in the tofersen group and −8.14 points in the placebo group (difference, 1.2 points; 95% confidence interval [CI], −3.2 to 5.5; P=0.97) (Table 2).

Secondary End Points in VALOR

Because statistical significance was not achieved for the primary end point, all subsequent differences between tofersen and placebo in the faster-progression subgroup are considered to be not significantly different, and no P values are presented. In the faster-progression subgroup, the total concentration of SOD1 protein in CSF was reduced by 29% in participants who received tofersen (geometric mean ratio to baseline, 0.71; 95% CI, 0.62 to 0.83), as compared with an increase of 16% (geometric mean ratio to baseline, 1.16; 95% CI, 0.96 to 1.40) in those who received placebo (between-group difference in geometric mean ratio, 0.62; 95% CI, 0.49 to 0.78) (Table 2). The total concentration of SOD1 protein in CSF was reduced by 40% in the tofersen-treated slower-progression subgroup, as compared with a reduction by 19% in the participants in the slower-progression subgroup who received placebo (between-group difference in geometric mean ratio, 0.74; 95% CI, 0.63 to 0.88) (Table S4). The mean concentration of neurofilament light chains in plasma was reduced by 60% in the tofersen-treated faster-progression subgroup and increased 20% with placebo (between-group difference in geometric mean ratio, 0.33; 95% CI, 0.25 to 0.45) (Table 2).

In the faster-progression subgroup, the percentage of predicted slow vital capacity declined by 14.3 points from baseline to week 28 among participants who received tofersen and declined by 22.2 points among those who received placebo (difference, 7.9 percentage points; 95% CI, −3.5 to 19.3) (Table 2). The change from baseline to week 28 in handheld dynamometry megascore was −0.34 in the tofersen group and −0.37 in the placebo group (difference, 0.02; 95% CI, −0.21 to 0.26). The median time to death or permanent ventilation could not be estimated owing to the small number of events; no difference was observed in the percentage of participants who died or required permanent ventilation in the tofersen group (10%) or in the placebo group (10%) (hazard ratio, 1.39; 95% CI, 0.22 to 8.80). The median time to death could not be estimated, with one event (3% of participants) in the tofersen group and no events in the placebo group (Table 2). Descriptive analyses in the slower-progression subgroup during VALOR are provided in Table S4.

COMBINED VALOR AND OPEN-LABEL EXTENSION

After completion of VALOR, 95 participants (88%) were enrolled in the nonrandomized open-label extension, with 63 (88%) originally assigned to receive tofersen and 32 (89%) originally assigned to receive placebo. At the time of the most recent data cutoff (January 16, 2022), 49 participants (68%) in the early-start cohort and 18 (50%) in the delayed-start cohort remained

Table 1. Demographic and Clinical Characteristics of the Participants at Baseline (Intention-to-Treat Population).*

Characteristic	Faster-Progression Subgroup (N=60)†		Slower-Progression Subgroup (N=48)		Overall (N=108)‡	
	Placebo (N=21)	Tofersen (N=39)	Placebo (N=15)	Tofersen (N=33)	Placebo (N=36)	Tofersen (N=72)
Age — yr	54.0±12.2	47.3±14.3	47.3±9.8	49.0±10.5	51.2±11.6	48.1±12.6
Male sex — no. (%)	11 (52)	22 (56)	8 (53)	21 (64)	19 (53)	43 (60)
Body-mass index§	28.0±6.2	26.7±6.4	26.6±7.0	26.2±4.6	27.4±6.5	26.4±5.6
Riluzole use — no. (%)	13 (62)	25 (64)	9 (60)	20 (61)	22 (61)	45 (62)
Edaravone use — no. (%)	1 (5)	2 (5)	2 (13)	4 (12)	3 (8)	6 (8)
Time from symptom onset — min¶						
Median (IQR)	8.3 (5.1 to 12.1)	8.3 (6.0 to 10.4)	39.6 (30.3 to 53.6)	35.5 (19.5 to 60.9)	14.6 (6.6 to 32.0)	11.4 (7.2 to 28.9)
Range	2.4 to 21.3	1.7 to 18.5	11.8 to 103.2	3.9 to 145.7	2.4 to 103.2	1.7 to 145.7
Concentration of neurofilament light chains in plasma — pg/ml						
Mean	127.3±94.4	146.2±82.6	37.0±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	9 to 370	12 to 329	8 to 99	5 to 211	8 to 370	5 to 329
ALSFRS-R prerandomization slope — points per mo‖						
Mean	−1.81±1.17	−1.74±1.58	−0.26±0.25	−0.30±0.20	−1.16±1.19	−1.08±1.37
Range	−4.91 to −0.42	−8.30 to −0.39	−0.84 to −0.02	−0.77 to 0.00	−4.91 to −0.02	−8.30 to 0.00
ALSFRS-R run-in slope — points per mo**						
Mean	−1.3±3.9	−1.8±2.5	0.1±1.9	−0.1±1.3	−0.7±3.3	−1.0±2.2
Range	−11 to 10	−9 to 3	−3 to 4	−3 to 4	−11 to 10	−9 to 4
ALSFRS-R total score						
Mean	35.4±5.7	36.0±6.4	39.9±5.1	38.1±5.1	37.3±5.8	36.9±5.9
Range	24 to 45	15 to 44	32 to 47	26 to 48	24 to 47	15 to 48
Percentage of predicted slow vital capacity						
Mean	83.7±17.9	80.3±14.2	87.1±14.8	84.2±19.0	85.1±16.5	82.1±16.6
Range	57.4 to 120.4	46.7 to 114.8	54.8 to 114.4	55.4 to 134.7	54.8 to 120.4	46.7 to 134.7

* Plus–minus values are means ±SD. Total scores on the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) range from 0 to 48, with higher scores indicating better function. IQR denotes interquartile range.
† These participants were included in the randomized VALOR component of the trial.
‡ These participants were included in the combined analysis.
§ The body-mass index is the weight in kilograms divided by the square of the height in meters.
¶ The time since the onset of symptoms of amyotrophic lateral sclerosis (ALS) was calculated in months as (date of baseline minus date of ALS symptom onset) divided by 30.4375.
‖ The prerandomization ALSFRS-R slope was calculated as (maximum possible score of 48 minus ALSFRS-R score at baseline [day 1]) divided by time since symptom onset.
** The run-in ALSFRS-R slope reflects the rate of decline in the ALSFRS-R score from screening to day 15 (run-in period of approximately 42 days).

Table 2. Primary and Secondary End Points in VALOR in the Faster-Progression Subgroup.*

End Point	Placebo (N=21)	Tofersen (N=39)
Primary end point		
ALSFRS-R total score†		
Adjusted mean change from VALOR baseline	–8.14	–6.98
Adjusted mean difference: tofersen minus placebo (95% CI)		1.2 (–3.2 to 5.5)
P value according to joint rank test and multiple imputation		0.97
Secondary end points		
Total SOD1 concentration in CSF		
Adjusted geometric mean ratio to VALOR baseline	1.16	0.71
Geometric mean ratio: tofersen vs. placebo (95% CI)		0.62 (0.49 to 0.78)
Concentration of neurofilament light chains in plasma		
Adjusted geometric mean ratio to VALOR baseline	1.20	0.40
Geometric mean ratio: tofersen vs. placebo (95% CI)		0.33 (0.25 to 0.45)
Percentage of predicted slow vital capacity — percentage points		
Adjusted mean change from VALOR baseline	–22.20	–14.31
Adjusted mean difference: tofersen minus placebo (95% CI)		7.9 (–3.5 to 19.3)
Handheld dynamometry megascore		
Adjusted mean change from VALOR baseline	–0.37	–0.34
Adjusted mean difference: tofersen minus placebo (95% CI)		0.02 (–0.21 to 0.26)
Death or permanent ventilation		
No. of events/total no. of participants (%)	2/21 (10)	4/39 (10)
Hazard ratio (95% CI)‡		1.39 (0.22 to 8.80)
Death		
No. of events/total no. of participants (%)	0/21	1/39 (3)
Hazard ratio (95% CI)‡		NE (NE to NE)

* Shown are the results at the end of the placebo-controlled period (week 28). CSF denotes cerebrospinal fluid, NE could not be estimated, and SOD1 superoxide dismutase 1.
† Sequential analysis failed at this point, and all subsequent secondary end points are considered to be not significantly different between trial groups.
‡ The hazard ratio is based on a Cox proportional-hazards model adjusted for baseline disease duration since symptom onset, baseline ALSFRS-R score, and use of riluzole or edaravone.

in the open-label extension. All 108 participants who underwent randomization in VALOR were included in the analysis of the combined data set for VALOR and the open-label extension, whether they were previously included as part of the faster-progression or slower-progression subgroup. In early-start participants, reductions in the total SOD1 concentration in CSF and the concentration of neurofilament light chains in plasma were numerically sustained over time; delayed-start participants had similar reductions during the open-label extension (Fig. 1). At 52 weeks, the change in the ALSFRS-R score from the VALOR baseline was –6.0 points for early-start participants and –9.5 points for delayed-start participants (difference, 3.5 points; 95% CI, 0.4 to 6.7). Imputation for week 52 was required for missing data in 15 participants (21%) in the early-start cohort and 8 participants (22%) in the delayed-start cohort (Fig. 2).

The change in the percentage of predicted slow vital capacity from the VALOR baseline was –9.4% for early-start participants and –18.6% for delayed-start participants (difference, 9.2 percentage points; 95% CI, 1.7 to 16.6). The change in handheld dynamometry megascore from the VALOR baseline was –0.17 for early-start participants and –0.45 for delayed-start participants

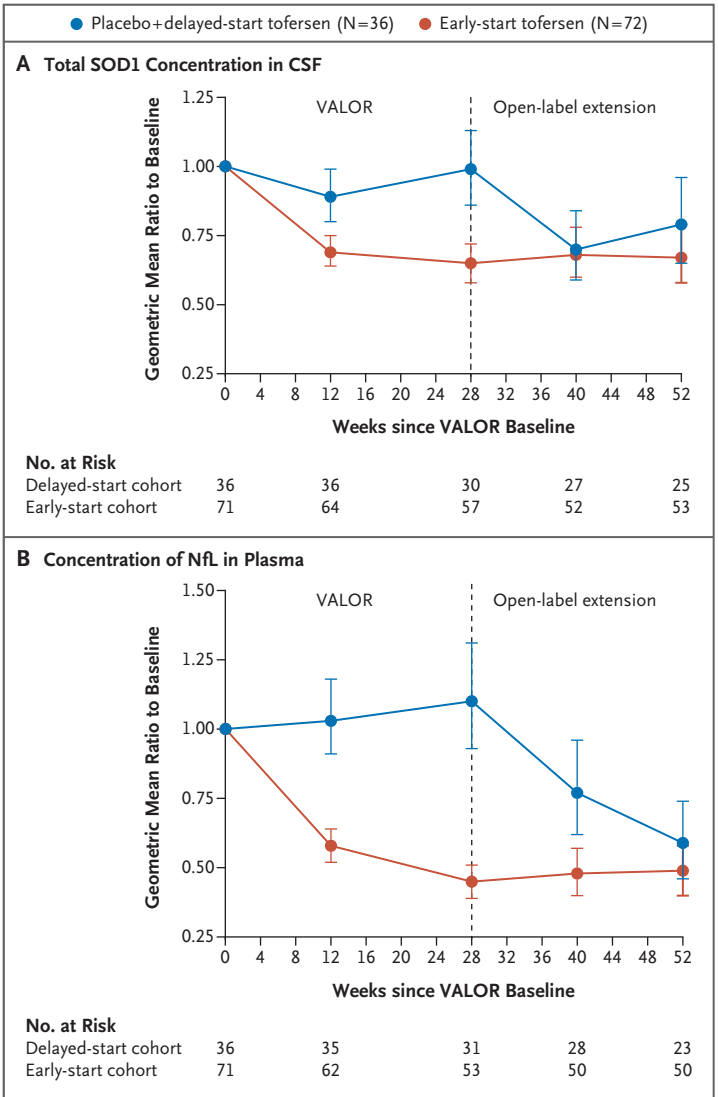


Figure 1. Total Superoxidase Dismutase 1 (SOD1) Concentrations in Cerebrospinal Fluid (CSF) and Concentrations of Neurofilament Light Chains (NfL) in Plasma.

Shown is the course of all randomly assigned participants (intention-to-treat population) over a period of 52 weeks, including those with faster-progressing disease and those with slower-progressing disease. After completing the 28-week placebo-controlled period, all the participants had the opportunity to receive tofersen in the open-label extension. Participants who initiated tofersen at week 0 in the VALOR component of the trial make up the "early-start cohort," and those who received placebo in VALOR and had the opportunity to cross over to tofersen in the open-label extension are referred to as the "delayed-start cohort." Participant disposition is summarized in Figure S3 in the Supplementary Appendix. I bars represent 95% confidence intervals.

(difference, 0.28; 95% CI, 0.05 to 0.52). Figure 2 and Table S5 show the results of the ALSFRS-R score, the percentage of predicted slow vital capacity, and the handheld dynamometry megascore for the combined analyses of VALOR and the open-label extension.

The median time to death or permanent ventilation and the median time to death could not be estimated owing to the limited number of events. For early-start participants as compared with delayed-start participants, the hazard ratio for time to death or permanent ventilation was 0.36 (95% CI, 0.14 to 0.94), and the hazard ratio for time to death was 0.27 (95% CI, 0.08 to 0.89) (Table S5). In a descriptive analysis, the disease duration in the 16 participants of special interest with p.Ala5Val mutations who received tofersen was a median of 1.73 years (range, 0.88 to 3.68), with 3 of these participants remaining in the trial at the time of the data cutoff (range for the 3 ongoing participants, 1.89 to 3.68 years) (Fig. S4).

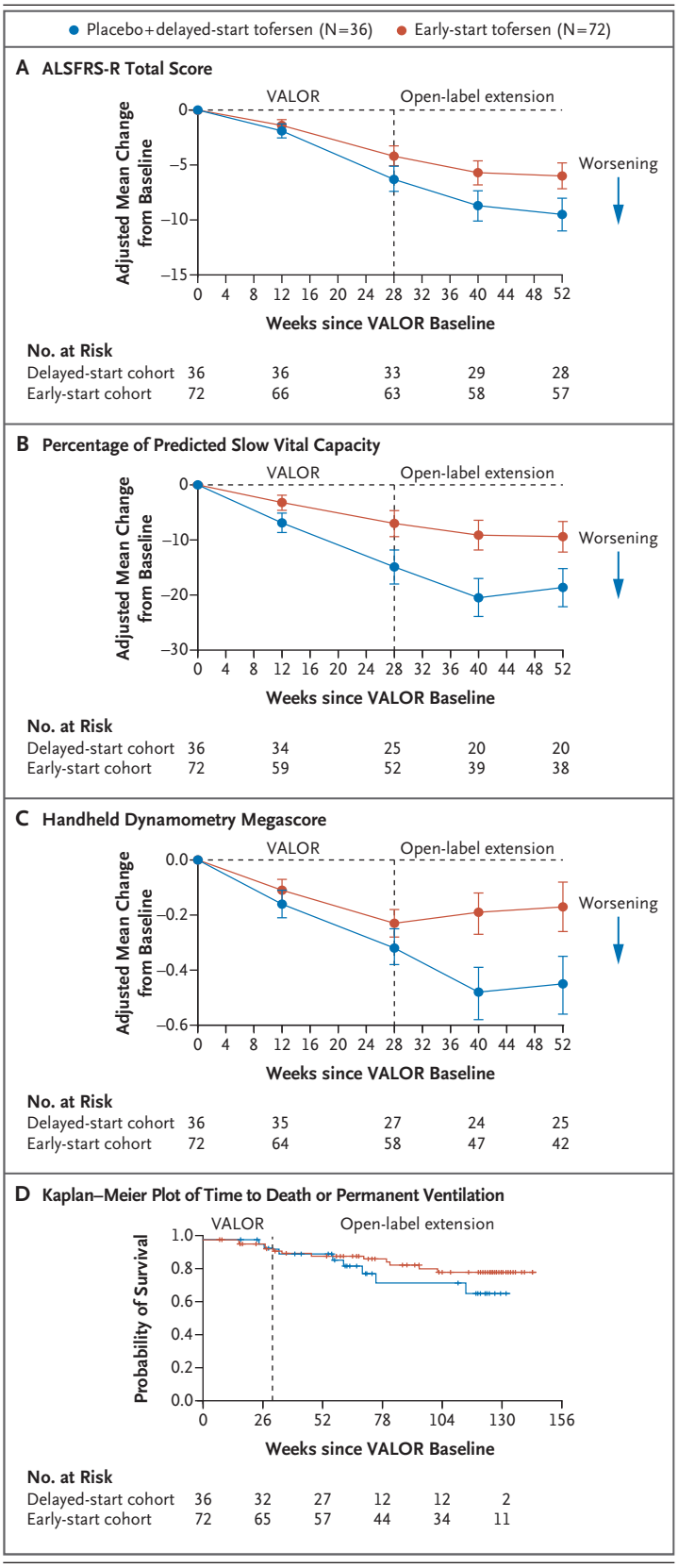
SAFETY AND ADVERSE EVENTS

Most adverse events across VALOR and the open-label extension were mild to moderate in severity and did not cause withdrawal or discontinuation of the trial agent. Most adverse events were consistent with ALS disease progression, conditions in the general population, or known side effects of lumbar puncture (Table 3). The most common adverse events included procedural pain, headache, pain in the arms or legs, falls, and back pain. In VALOR, the incidence of procedural pain and headache were similar among participants who received tofersen and among those who received placebo, whereas pain in the arms or legs and back pain were more common in the tofersen group (incidence higher by ≥5 percentage points) and falls were more common in the placebo group.

Four participants who received tofersen in VALOR (6%) and three participants in the open-label extension (constituting 7% of all participants who received tofersen) had a total of eight neurologic serious adverse events, including myelitis, chemical or aseptic meningitis, lumbar radiculopathy, increased intracranial pressure, and papilledema. The participant with myelitis was hospitalized approximately 1 week after the fifth dose of tofersen, received glucocorticoids and plasma exchange, and received no further trial treatment. Within 3 months after the last dose of tofersen, this participant had resolution of neurologic signs, symptoms, and findings on imaging.

Figure 2. Analyses of Clinical Function and Survival.

Shown is the course of all randomly assigned participants (intention-to-treat population). Panels A, B, and C reflect data over a period of 52 weeks since VALOR baseline. Panel D reflects all available follow-up data from VALOR baseline to the data-cutoff date of the open-label extension (January 16, 2022). After completing the 28-week placebo-controlled period, all the participants had the opportunity to receive tofersen in the open-label extension. Participants who initiated tofersen at week 0 in the VALOR component of the trial make up the "early-start cohort," and those who received placebo in VALOR and had the opportunity to cross over to tofersen in the open-label extension are referred to as the "delayed-start cohort." Participant disposition is summarized in Figure S3. Numerical results at 52 weeks were not adjusted for multiple comparisons. Total scores on the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) (Panel A) range from 0 to 48, with higher scores indicating better function. The handheld dynamometry megascore (Panel C) is the average of z-scores across 16 muscle groups in the arms and legs, with higher values indicating greater strength. I bars represent standard errors.



In VALOR, 42 participants (58%) in the tofersen group and 2 participants (6%) in the placebo group had at least one CSF white-cell count of more than 10 cells per cubic millimeter, and approximately 40% of the participants had elevated CSF protein concentrations at baseline. The median CSF protein concentration increased by 110 mg per liter in the tofersen group and decreased by 15 mg per liter in the placebo group. Similar incidences of CSF pleocytosis and elevated protein concentrations were observed during the open-label extension.

DISCUSSION

In the 28-week randomized VALOR component of the trial, tofersen was associated with reductions in the total concentration of SOD1 protein in CSF, an indirect marker of target engagement, and the concentration of neurofilament light chains in plasma, a marker of axonal injury and neurodegeneration. Despite these results, no significant difference was seen at 28 weeks in the change from baseline in the ALSFRS-R score between tofersen and placebo in a subgroup predicted to have faster progression, and no definitive differences were seen in other clinical end points in this subgroup. At 52 weeks in a prespecified combined analysis of VALOR and

Table 3. Summary of Adverse Events.*

Adverse Event	VALOR		VALOR and Open-Label Extension Combined†
	Placebo (N=36)	Tofersen (N=72)†	Tofersen (N=104)
	number of participants (percent)‡		
Any event	34 (94)	69 (96)	102 (98)
Event related to trial agent§	2 (6)	28 (39)	63 (61)
Event related to lumbar puncture§	29 (81)	58 (81)	84 (81)
Serious event	5 (14)	13 (18)	38 (37)
Serious event related to trial agent§	0	4 (6)	7 (7)
Event with fatal outcome	0	1 (1)	14 (13)
Events leading to discontinuation of trial agent	0	4 (6)	18 (17)
Adverse events occurring in ≥15% of participants in combined analysis¶			
Headache	16 (44)	33 (46)	58 (56)
Procedural pain	21 (58)	41 (57)	56 (54)
Fall	15 (42)	17 (24)	40 (38)
Back pain	2 (6)	15 (21)	39 (38)
Pain in arm or leg	6 (17)	19 (26)	38 (37)
Arthralgia	2 (6)	10 (14)	28 (27)
CSF protein concentration increased	1 (3)	6 (8)	24 (23)
Fatigue	2 (6)	12 (17)	24 (23)
Post–lumbar puncture syndrome	11 (31)	13 (18)	22 (21)
Myalgia	2 (6)	10 (14)	21 (20)
CSF white-cell count increased	0	7 (10)	19 (18)
Nausea	6 (17)	9 (12)	17 (16)
Constipation	4 (11)	6 (8)	17 (16)
Pyrexia	1 (3)	3 (4)	16 (15)
Serious adverse events occurring in ≥2% of participants in combined analysis¶			
Respiratory failure	0	1 (1)	10 (10)
Pneumonia aspiration	0	2 (3)	9 (9)
Pulmonary embolism	1 (3)	3 (4)	4 (4)
Acute respiratory failure	0	1 (1)	4 (4)
Dysphagia	0	0	3 (3)

* Shown are events that had an onset date on or after the start of the trial agent or that worsened after the start of the trial agent.
† An event in a participant who received placebo during VALOR is counted only once. An event in a participant who received tofersen during VALOR is counted in both columns for tofersen.
‡ A participant could appear in more than one category.
§ The relatedness of an event to the trial agent or lumbar puncture was assessed by the investigator.
¶ A participant is counted only once for each preferred term (*Medical Dictionary for Regulatory Activities*, version 24.0).

its open-label extension, participants who started tofersen at the beginning of VALOR, irrespective of fast or slow progression, had a smaller numeric decline in the ALSFRS-R score, the percentage of predicted slow vital capacity, and handheld dynamometry megascore than those

who started tofersen in the open-label extension 28 weeks later. Limitations in interpreting the results of the combined analysis include the absence of adjustment of the widths of confidence intervals for multiple comparisons in the analysis of differences between the early-start and delayed-start cohorts, approximately 20% of missing end-point data that required imputation, and the results of the VALOR component of the trial being known at the time of analysis.

Neurologic serious adverse events, including myelitis, chemical or aseptic meningitis, lumbar radiculopathy, increased intracranial pressure, and papilledema, occurred in approximately 7% of the participants receiving tofersen. The underlying mechanism of myelitis and the relationship to CSF pleocytosis and protein elevations could not be established.

At the time that the trial was designed, *SOD1* mutation type and prerandomization ALSFRS-R slope were considered to be appropriate tools for addressing the heterogeneity of disease progression in *SOD1* ALS, but neither is consistently prognostic over a short trial period. Although the prognostic usefulness of neurofilament light chains had been characterized at that time, assay limitations precluded randomization according to an individual participant's baseline concentration of neurofilament light chains, which would have enabled better balance across trial groups. Instead, subgroup analyses were prespecified in our trial and defined according to the median baseline concentration of neurofilament light chains. This approach helped to address imbalances in baseline characteristics (concentration of neurofilament light chains in plasma and ALSFRS-R decline from screening to day 15) but made use of arbitrary subgrouping rather than controlling for each participant's baseline concentration of neurofilament light chains (see Section S2 in the Supplementary Appendix). To address individual disease heterogeneity, the baseline concentration of neurofilament light chains in plasma was incorporated as a covariate across analyses. This alteration to the analysis plan was specified after the VALOR results and initial results from the combined VALOR and open-label extension were available but before the latest combined analysis was conducted. As testing of neurofilament light chains becomes more readily available, randomization based on the concentration of neurofilament light chains in plasma as a continuous variable may be considered in future ALS clinical trials.

The duration and size of VALOR were determined on the basis of available but limited data from 12 *SOD1* mutation carriers with rapidly progressing disease who received placebo in the tofersen phase 1–2 multiple-ascending-dose study[15] and the phase 2 trial of arimoclomol, a heat-shock protein coinducer that promotes nascent protein folding.[25] These persons had a rapid decline in function over the period of these studies. In contrast, the participants who received placebo in the enriched faster-progression subgroup in VALOR had declines that were three times as slow as those projected by the data.

The possible signal of differences in clinical end points between the early-start and delayed-start cohorts in the combined analysis of VALOR and the open-label extension, with the limitations mentioned, suggests that a trial duration of more than 28 weeks may be required to determine the effect of tofersen in patients with this disorder.[26,27] Earlier or presymptomatic intervention is being investigated in the ongoing ATLAS trial (ClinicalTrials.gov number, NCT04856982).[28]

In the 28-week VALOR component of this trial of intrathecal administration of the antisense oligonucleotide tofersen in patients with *SOD1* ALS, there was not a significant difference in the decline on a composite measure of ALS progression as compared with placebo. Tofersen was associated in a limited number of participants with adverse events, including myelitis. The potential effects of earlier as compared with delayed initiation of tofersen are being further evaluated in the ongoing extension phase.

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APPENDIX

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