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Phase 1–2 Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS

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ABSTRACT

BACKGROUND

Tofersen is an antisense oligonucleotide that mediates the degradation of superoxide dismutase 1 (SOD1) messenger RNA to reduce SOD1 protein synthesis. Intrathecal administration of tofersen is being studied for the treatment of amyotrophic lateral sclerosis (ALS) due to *SOD1* mutations.

METHODS

We conducted a phase 1–2 ascending-dose trial evaluating tofersen in adults with ALS due to *SOD1* mutations. In each dose cohort (20, 40, 60, or 100 mg), participants were randomly assigned in a 3:1 ratio to receive five doses of tofersen or placebo, administered intrathecally for 12 weeks. The primary outcomes were safety and pharmacokinetics. The secondary outcome was the change from baseline in the cerebrospinal fluid (CSF) SOD1 concentration at day 85. Clinical function and vital capacity were measured.

RESULTS

A total of 50 participants underwent randomization and were included in the analyses; 48 participants received all five planned doses. Lumbar puncture–related adverse events were observed in most participants. Elevations in CSF white-cell count and protein were reported as adverse events in 4 and 5 participants, respectively, who received tofersen. Among participants who received tofersen, one died from pulmonary embolus on day 137, and one from respiratory failure on day 152; one participant in the placebo group died from respiratory failure on day 52. The difference at day 85 in the change from baseline in the CSF SOD1 concentration between the tofersen groups and the placebo group was 2 percentage points (95% confidence interval [CI], –18 to 27) for the 20-mg dose, –25 percentage points (95% CI, –40 to –5) for the 40-mg dose, –19 percentage points (95% CI, –35 to 2) for the 60-mg dose, and –33 percentage points (95% CI, –47 to –16) for the 100-mg dose.

CONCLUSIONS

In adults with ALS due to *SOD1* mutations, CSF SOD1 concentrations decreased at the highest concentration of tofersen administered intrathecally over a period of 12 weeks. CSF pleocytosis occurred in some participants receiving tofersen. Lumbar puncture–related adverse events were observed in most participants. (Funded by Biogen; ClinicalTrials.gov number, NCT02623699; EudraCT number, 2015-004098-33.)

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Up to 2% of all cases of amyotrophic lateral sclerosis (ALS) result from mutations in the gene encoding superoxide dismutase 1 (*SOD1*).[1,2] More than 180 different *SOD1* mutations have been identified in ALS.[3-5] Disease penetration, progression rate, and prognosis are variable and may be mutation-specific.[6,7]

The mechanisms by which mutations in *SOD1* cause degeneration of motor neurons in genetic ALS are not fully understood; a toxic gain of function has been considered to be the most likely mechanism in ALS caused by *SOD1* mutations.[1,8-13] Lowering the concentration of mutant SOD1 protein may be a potential target for therapeutic intervention.[6,14] Antisense oligonucleotides (ASOs) have been generally safe for the treatment of other diseases, including spinal muscular atrophy.[15,16] ASOs targeting SOD1 messenger RNA (mRNA) transcripts prolonged survival and improved motor performance in rodent models of ALS caused by *SOD1* mutations and reduced SOD1 protein concentrations in nonhuman primates.[9]

Tofersen (BIIB067) is an ASO that is under investigation for the treatment of ALS caused by *SOD1* mutations. Tofersen has been designed to mediate RNase H–dependent degradation of SOD1 mRNA to reduce the synthesis of SOD1 protein.[9,17,18] We are conducting a three-part trial: parts A (single ascending dose)[19] and B (multiple ascending dose) have been completed, and part C is ongoing. Here, we describe the results from part B, a multiple ascending-dose trial that evaluated the safety, pharmacokinetics, and pharmacodynamics of tofersen in adults with ALS.

Methods

Oversight

We conducted this trial in accordance with the Good Clinical Practice guidelines of the International Council for Harmonisation, and the trial protocol (available with the full text of this article at NEJM.org) was approved by the relevant ethics committees. There was no data and safety monitoring board for this portion of the trial. Written informed consent was provided by the participants or their legal representatives.

The sponsor (Biogen) and the academic authors designed the trial. Biogen provided tofersen and placebo and oversaw the trial. Data were collected by the investigators and analyzed by the sponsor. The first draft of the manuscript was written by the first author and the senior industry author; medical writing assistance was paid for by the sponsor. The sponsor reviewed the manuscript and provided feedback to the authors. The authors had full editorial control of the manuscript and provided their final approval of all content. All the authors vouch for the accuracy and completeness of the data, for the fidelity of the trial to the protocol, and for the complete reporting of adverse events. Confidentiality agreements were in place between the authors and the sponsor.

Participants

The trial included adults who had muscle weakness that had been attributed to ALS on the basis of conventional criteria and a documented *SOD1* mutation. Inclusion criteria included normal coagulation variables and a forced vital capacity of at least 50% of the predicted value as adjusted for sex, age, and height. Participants who were receiving riluzole were required to maintain a stable dose, whereas patients who were receiving edaravone or were anticipated to receive edaravone were excluded from the trial. (Riluzole and edaravone are drugs used in the treatment of ALS). Full details of the inclusion and exclusion criteria are provided in the protocol.

Trial Design

This phase 1–2, randomized, double-blind, placebo-controlled trial was conducted at 18 sites in the United States, Canada, and four countries in Western Europe (Belgium, France, Germany, and the United Kingdom) beginning in January 2016 (Fig. S1 in the Supplementary Appendix, available at NEJM.org). The participating investigators are listed according to country in Section S1 in the Supplementary Appendix. Participants were followed for up to 31 weeks, which comprised a screening period of up to 7 weeks followed by a 12-week intervention period and a 12-week follow-up (Fig. S1).

Participants were assigned to one of four dose cohorts (20, 40, 60, or 100 mg), which were assessed sequentially. Progression of the trial to the next dose-level cohort was based on a blinded review of safety and pharmacokinetic data from the preceding cohorts by the sponsor and the investigators. Each dose-level cohort included 12

participants who had been randomly assigned by means of interactive response technology in a 3:1 ratio to receive tofersen or placebo (consisting of artificial cerebrospinal fluid [CSF]) as a lumbar intrathecal bolus injection; details of the injection method are provided in the protocol. If a participant withdrew from the trial before completing the final follow-up visit, the sample size could be increased by 1 at the discretion of the sponsor. No participants would be removed from analyses. All participants who withdrew and those who completed the trial would be included in the analyses. A single dose of tofersen or placebo was administered on days 1, 15, 29, 57, and 85 (Fig. S1).

OUTCOMES

Primary outcomes were the incidence of adverse events and serious adverse events; abnormalities in clinical laboratory assessments and vital signs; physical examination including cranial nerves, coordination and cerebellar function, reflexes, motor function, and Mini–Mental State Examination; electrocardiograms; and tofersen pharmacokinetic measures in plasma and CSF. The secondary outcome was the change from baseline in the SOD1 protein concentration in CSF at day 85. The concentration was assessed with the use of a commercially available enzyme-linked immunosorbent assay kit that was qualified by the contract research organization (Covance) for quantification of SOD1 protein in CSF (Thermo Fisher Scientific).

Exploratory outcomes included changes from baseline in the total score on the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R; 12 items in four domains of function are measured, each scored on a scale from 0 to 4, with higher scores indicating better function), the percentage of the predicted slow vital capacity, the handheld dynamometry megascore (which assesses strength in 16 muscle groups in the arms and legs; z-score normalization is applied to the scores, with lower scores indicating worse function), and neurofilament concentrations (phosphorylated neurofilament heavy chains and a post hoc analysis of neurofilament light chains) (Section S2). A subgroup of participants with fast-progressing disease was defined post hoc for further analysis as described in the Statistical Analysis subsection, below. One staff member at each site consistently performed the ALSFRS-R and remained unaware of the trial-group assignments and the results of other assessments. The concentrations of neurofilament light chains were assessed with the use of the Simoa NF-light Advantage assay (Quanterix), and concentrations of phosphorylated neurofilament heavy chains with the use of the Simple Plex Ella immunoassay (ProteinSimple).

STATISTICAL ANALYSIS

A 25% reduction in the CSF SOD1 protein concentration was estimated to exceed both the SOD1 assay variability and the longitudinal biovariability of CSF SOD1 as measured in humans. We calculated that 12 participants (9 to receive tofersen and 3 to receive placebo) in each of the four dose cohorts would be needed for the trial to have at least 80 to 99% power to detect differences in the reduction in the CSF SOD1 concentration (tofersen vs. placebo) between 12% and 25% at a 10% significance level. This sample-size calculation assumed a pooled standard deviation of 0.11 (natural log). The interim analysis that is described in the statistical analysis plan (available with the protocol) was conducted on all data up to the day 85 visit for 11 of the 12 participants in the 100-mg dose cohort to permit planning for the phase 3 trial (the 12th participant had not competed day 85 of the study). The results presented here reflect data from the final analysis.

The modified intention-to-treat population, which was defined as all the participants who had undergone randomization and received at least part of one dose of tofersen or placebo, was also the safety population. Assessments of clinical function, pharmacokinetics, pharmacodynamics, and biomarkers included participants who had at least one relevant measurement after receipt of the dose.

Safety, clinical, pharmacokinetic, pharmacodynamic, and exploratory outcomes were summarized according to trial visit and according to dose with the use of descriptive statistics. Participants who received placebo were combined across all cohorts for all summaries and analyses. Postbaseline missing measurements for the SOD1 concentration, ALSFRS-R, the slow vital capacity, and the neurofilament concentrations were imputed with the use of a mixed model for re-

Table 1. Adverse Events Occurring in at Least Three Participants in Any Group.*

Event	Placebo (N=12)	Tofersen, 20 mg (N=10)	Tofersen, 40 mg (N=9)	Tofersen, 60 mg (N=9)	Tofersen, 100 mg (N=10)
	number of participants (percent)				
Any adverse event	12 (100)	10 (100)	9 (100)	9 (100)	10 (100)
Headache	7 (58)	4 (40)	2 (22)	4 (44)	6 (60)
Procedural pain	5 (42)	4 (40)	1 (11)	4 (44)	7 (70)
Post–lumbar puncture syndrome	3 (25)	4 (40)	3 (33)	3 (33)	3 (30)
Fall	3 (25)	3 (30)	3 (33)	2 (22)	5 (50)
Back pain	0	1 (10)	1 (11)	1 (11)	5 (50)
Nasopharyngitis	1 (8)	2 (20)	1 (11)	3 (33)	1 (10)
Upper respiratory tract infection	0	4 (40)	0	2 (22)	0
CSF protein concentration increased	1 (8)	0	0	4 (44)	1 (10)
CSF white-cell count increased	0	0	1 (11)	3 (33)	0
Pain in arm or leg	2 (17)	0	1 (11)	0	3 (30)
Dizziness	3 (25)	0	0	0	1 (10)
Neck pain	3 (25)	0	0	1 (11)	0

* The placebo group includes all the participants who had been assigned to receive placebo in any dose-matched cohort. CSF denotes cerebrospinal fluid.

peated measures; summary statistics for the handheld dynamometry megascore were calculated on the basis of the last observation carried forward (Section S3). All the outcomes are reported with descriptive statistics, without P values; results are presented as point estimates with 95% confidence intervals that were not adjusted for multiplicity, and therefore no inferences can be made. For slow vital capacity, the value associated with the maximum (best) effort at the visit was analyzed, regardless of acceptability according to American Thoracic Society guidelines. All the statistical analyses were performed with SAS software, version 9.4 or higher (SAS Institute).

A fast-progression subgroup was defined post hoc for the purpose of analysis. Participants in the fast-progression subgroup had a *SOD1* mutation that was characterized as involving a fast-progressing disease course (from a prespecified list of 10 mutations associated with a mean disease duration of ≤3 years) (Section S4 and Table S1), in addition to having a decrease in the prerandomization slope of the ALSFRS-R score of at least 0.2 points per month. Participants who did not meet this definition for fast-progressing disease were included in the "other" subgroup for post hoc analysis.

RESULTS

CHARACTERISTICS OF THE PARTICIPANTS

From November 7, 2016, to July 17, 2018, a total of 55 participants underwent screening, and 50 were enrolled in the trial (Fig. S2). (We increased the sample size from 48 to 50 to account for 2 participants who withdrew during the intervention period; 1 participant in the placebo group died, and 1 in the 20-mg dose group withdrew consent and was then lost to follow-up.) A total of 12 participants were assigned to receive placebo across the dose cohorts; among participants assigned to receive tofersen, 10 were assigned to the 20-mg dose group, 9 to the 40-mg dose group, 9 to the 60-mg dose group, and 10 to the 100-mg dose group. Two participants received an initial dose in a single-dose study (part A of this study) and subsequently enrolled in the current multiple-dose trial after washout periods of 32 weeks and 42 weeks. All the participants had a documented *SOD1* mutation, reported limb paresis at the onset of the illness, and received at least one dose of tofersen or placebo. Two participants who discontinued the trial were replaced. A total of 48 participants received all five planned doses. Participants who met the definition for

Table 2. Summary of Overall Adverse Events.*

Event	Placebo (N=12)	Tofersen, 20 mg (N=10)	Tofersen, 40 mg (N=9)	Tofersen, 60 mg (N=9)	Tofersen, 100 mg (N=10)
	number of participants (percent)				
Grade†					
1: mild	5 (42)	3 (30)	4 (44)	4 (44)	4 (40)
2: moderate	3 (25)	5 (50)	4 (44)	2 (22)	6 (60)
3: severe or medically significant	3 (25)	1 (10)	1 (11)	2 (22)	0
4: life-threatening	0	0	0	0	0
5: death related to adverse event	1 (8)	1 (10)	0	1 (11)	0
Event related to placebo or tofersen‡	0	2 (20)	2 (22)	6 (67)	4 (40)
Event related to lumbar puncture‡	9 (75)	8 (80)	7 (78)	8 (89)	9 (90)
Serious event	2 (17)	2 (20)	1 (11)	2 (22)	0
Serious event related to placebo or tofersen‡	0	0	0	1 (11)	0
Serious event leading to discontinuation of placebo or tofersen	1 (8)	0	0	0	0
Serious event leading to interruption of trial regimen	0	0	0	0	0
Serious event leading to withdrawal from trial	1 (8)	1 (10)	0	1 (11)	0
Death	1 (8)	1 (10)	0	1 (11)	0

* The severity of adverse events and serious adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4. Participants may have had more than one event in each category. The placebo group includes all the participants who had been assigned to receive placebo in any dose-matched cohort.
† Each participant was counted once at the maximum grade of adverse event.
‡ Events related to placebo or tofersen (as determined in a blinded fashion), lumber puncture, or both were determined by the trial investigator.

the fast-progression subgroup were identified post hoc: 1 participant each at the tofersen dose levels of 20 mg and 40 mg, 2 participants at the 60-mg dose level, and 4 participants each at the 100-mg dose level and in the placebo group.

The characteristics of the participants at baseline that were related to disease progression (time since treatment onset and prerandomization slope of ALSFRS-R score) were balanced in the 100-mg dose group and the placebo group, a finding that was consistent with the proportion of participants with fast progression in each cohort. However, the characteristics of the participants at baseline were not well balanced between the other tofersen dose groups and the placebo group. Regarding baseline characteristics other than those related to disease progression, the placebo group had a higher proportion of men, a lower percentage of participants receiving riluzole, a lower percentage of the predicted slow vital capacity, a higher handheld dynamometry megascore, and a lower SOD1 concentration in CSF than the 100-mg dose group (Table S2).

SAFETY AND ADVERSE EVENTS

All 50 participants were included in the safety analyses, and all the participants reported having one or more adverse events. The most common adverse events among the 38 participants who received one or more doses of tofersen were headache (in 16 participants), procedural pain (in 16), post–lumbar puncture syndrome (in 13), and falls (in 13) (Table 1).

Five participants who received tofersen and two who received placebo had serious adverse events (Table 2). Three deaths occurred: one in the placebo group during the intervention period (respiratory failure related to ALS on day 52), one in the 20-mg dose group during follow-up (pulmonary embolism on day 137), and one in the 60-mg dose group during follow-up (respiratory failure related to ALS on day 152). One par-

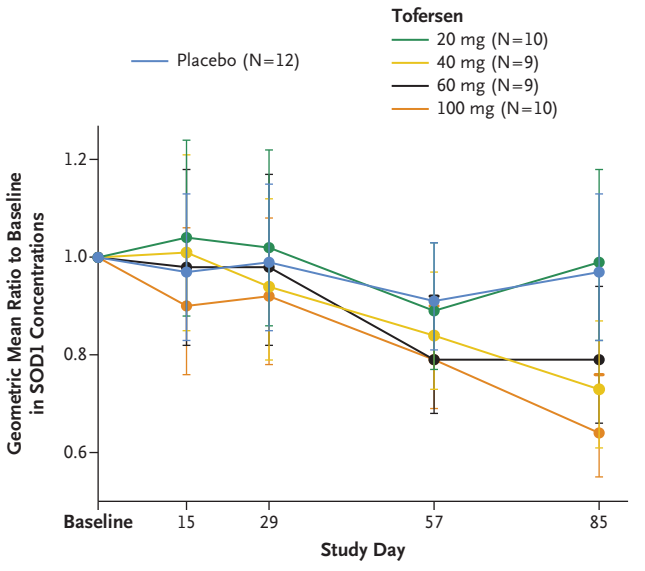

Figure 1. Effect of Tofersen Treatment on Total Superoxide Dismutase 1 (SOD1) Protein Concentrations in Cerebrospinal Fluid.

The geometric mean ratio between the baseline value and the values at the specified time points are shown. Geometric mean ratios were calculated with the use of the least-squares method. I bars indicate 95% confidence intervals. In the combined placebo group, there was one anomaly for a cerebrospinal fluid sample obtained at day 15; the result was below the limit of quantitation and was noted as being missing data. All missing data were imputed with the use of a mixed model for repeated measures.

ticipant withdrew consent after receiving two doses of tofersen (in the 20-mg dose group) and discontinued the trial owing to disease progression prohibiting travel, and one participant in the placebo group withdrew consent after completing the intervention period.

At baseline and during the trial, an elevated CSF protein concentration or CSF pleocytosis (or both) was observed. In the combined placebo group, 1 of 12 participants (8%) had at least one CSF white-cell count of more than 10 cells per cubic millimeter, as compared with 16 of 38 participants (42%) in the combined tofersen groups, with a maximum observed value of 144 white cells per cubic millimeter. Elevations in CSF white-cell count and protein were reported as adverse events in 4 and 5 participants, respectively, who received tofersen; 1 participant who received placebo had an adverse event of elevated CSF protein concentration. There was no clear association between higher doses of tofersen or placebo or longer duration of exposure and greater CSF pleocytosis or elevated protein concentrations (Fig. S3). None of these adverse events or CSF abnormalities (that were not considered to be adverse events) led to trial discontinuation. Pharmacokinetic data are summarized in Section S5 and Figure S4.

SOD1 REDUCTION IN CSF

The changes from baseline in the total SOD1 protein concentrations in CSF are presented in Figure 1. The geometric mean SOD1 concentration in CSF at baseline among participants who received tofersen was 79.9 ng per milliliter in the 20-mg dose group, 140.9 ng per milliliter in the 40-mg dose group, 102.5 ng per milliliter in the 60-mg dose group, and 139.8 ng per milliliter in the 100-mg dose group; among participants who received placebo, the concentration was 84.6 ng per milliliter. The geometric mean ratios of the SOD1 protein concentrations among participants who received tofersen decreased from baseline to day 85 by 1% in the 20-mg dose group, by 27% in the 40-mg dose group, by 21% in the 60-mg dose group, and by 36% in the 100-mg dose group; among participants who received placebo, the ratio decreased by 3%. The difference in the ratio between the geometric mean value at day 85 and the baseline value of CSF SOD1 concentration between the tofersen groups and the placebo group was 2 percentage points (95% confidence interval [CI], −18 to 27) for the 20-mg dose group, −25 percentage points (95% CI, −40 to −5) for the 40-mg dose group, −19 percentage points (95% CI, −35 to 2) for the 60-mg dose group, and −33 percentage points (95% CI, −47 to −16) for the 100-mg dose group (Table S3). The baseline concentrations and the magnitude of SOD1 reduction in CSF were generally similar in the fast-progression subgroup and in the participants in the 100-mg dose group who were in the other subgroup. In an analysis that included only the 36 participants across all the tofersen groups and the placebo group who completed the trial to day 169, the geometric mean SOD1 concentrations in CSF were higher at day 169 than at day 85 (Fig. S5).

EXPLORATORY OUTCOMES

Results regarding clinical function (as assessed by the total ALSFRS-R score), respiratory function (as assessed by the percent predicted slow vital capacity), and muscle strength (as assessed by the handheld dynamometry megascore) are shown

Table 3. Clinical Measures of Change in Clinical Function, Respiratory Function, and Muscle Strength.*

Outcome	Placebo (N=12)	Tofersen, 20 mg (N=10)	Tofersen, 40 mg (N=9)	Tofersen, 60 mg (N=9)	Tofersen, 100 mg (N=10)
Least-squares mean change in ALSFRS-R score (95% CI) — points					
At day 15	−1.11 (−2.17 to −0.05)	−0.34 (−1.59 to 0.91)	−0.46 (−1.75 to 0.83)	−0.40 (−1.66 to 0.86)	−1.13 (−2.29 to 0.02)
At day 29	−1.29 (−2.88 to 0.30)	−0.88 (−2.73 to 0.96)	−0.69 (−2.56 to 1.19)	−0.82 (−2.68 to 1.04)	−1.91 (−3.65 to 0.17)
At day 57	−4.50 (−7.21 to −1.78)	−0.97 (−4.09 to 2.15)	−1.35 (−4.44 to 1.74)	−1.97 (−5.06 to 1.13)	−2.24 (−5.16 to 0.67)
At day 85	−5.63 (−8.90 to −2.36)	−0.76 (−4.49 to 2.97)	−0.82 (−4.50 to 2.85)	−2.13 (−5.82 to 1.56)	−1.19 (−4.67 to 2.29)
Least-squares mean change in percentage of predicted slow vital capacity (95% CI) — percentage points					
At day 15	−1.21 (−6.66 to 4.25)	0.15 (−5.99 to 6.29)	−2.06 (−8.86 to 4.74)	1.72 (−4.28 to 8.22)	−7.52 (−13.41 to −1.62)
At day 29	−4.88 (−9.29 to −0.47)	0.23 (−4.95 to 5.42)	−1.65 (−7.11 to 3.81)	−0.98 (−6.47 to 4.50)	−2.50 (−7.17 to 2.18)
At day 57	−14.00 (−19.85 to −8.15)	−3.48 (−10.22 to 3.25)	−0.03 (−7.24 to 7.18)	−2.28 (−4.69 to 9.25)	−3.06 (9.32 to 3.20)
At day 85	−14.46 (−21.79 to −7.12)	−9.17 (−17.24 to −1.10)	−6.30 (−15.01 to 2.41)	−0.18 (−8.54 to 8.19)	−7.08 (−14.69 to 0.54)
Mean change in handheld dynamometry megascore — points†					
At day 22	−0.14±0.27	NA	−0.06±0.23	−0.10±0.10	0.17±0.56
At day 92	−0.26±0.42	−0.14±0.20	−0.02±0.30	−0.16±0.26	−0.03±0.18

* Plus–minus values are means ±SD. Clinical function was assessed with the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R), which measures 12 items in four domains of function, each scored on a scale from 0 to 4, with higher scores indicating better function. Respiratory function was assessed as the percentage of the predicted slow vital capacity. Muscle strength was assessed with the handheld dynamometry megascore, which assesses strength in 16 muscle groups in the arms and legs; z-score normalization was applied to scores, with lower scores indicating worse function. Changes in the ALSFRS-R score and the percentage of the predicted slow vital capacity are shown as least-squares means, and changes in the handheld dynamometry megascore are raw means. Because of the absence of a plan for adjustment for multiple comparisons, point estimates and multiplicity unadjusted confidence intervals are presented, from which no inferences can be made. NA denotes not available.

† Because of the timing of the protocol amendment to obtain the handheld dynamometry megascore at day 22, no participants in cohort 5 (in which participants received placebo or 20 mg of tofersen) had data for this score at this visit. Data were available for nine participants in the placebo group (i.e., those in other dose-matched cohorts) and for no participants in the 20-mg dose group. At day 22, data were available for eight participants in the 40-mg dose group; at day 92, data were available for nine participants in the 20-mg dose group.

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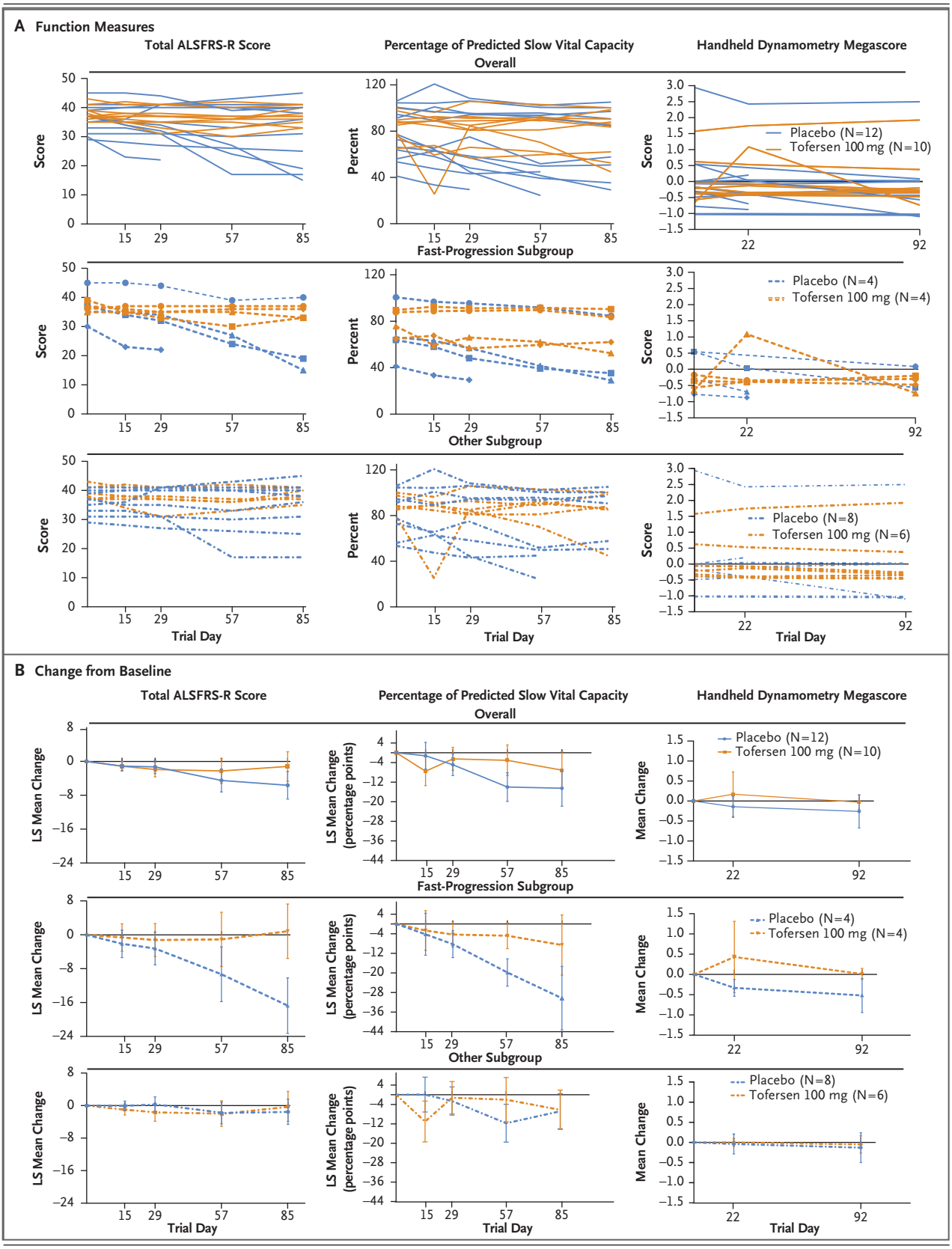

Figure 2 (facing page). Change from Baseline and Individual Traces for the Total Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) Score, the Percentage of Predicted Slow Vital Capacity, and Handheld Dynamometry Megascore.

Panel A shows individual values for the ALSFRS-R scores, the percentage of the predicted slow vital capacity, and handheld dynamometry megascores for all participants over time. The ALSFRS-R measures 12 items in four domains of function, each scored on a scale from 0 to 4, with higher scores indicating better function. Handheld dynamometry measures strength in 16 muscle groups in the arms and legs; z-score normalization was applied to scores, with lower scores indicating worse function. Postbaseline missing values were imputed with the use of a mixed model for repeated measures for the ALSFRS-R score and the percentage of the predicted slow vital capacity; the last-observation-carried-forward method was used for the handheld dynamometry megascore. Baseline values were not carried forward, which resulted in missing data at day 22 for three participants in the placebo group (one in the fast-progression subgroup and two in the other subgroup, which included participants who did not meet the criteria for fast-progressing disease). The two subgroups were defined post hoc, and no conclusions can be drawn from these data. Panel B shows the least-squares (LS) mean total ALSFRS-R scores, the LS mean percentage of the predicted slow vital capacity, and the raw mean handheld dynamometry megascore in the 100-mg dose group and the placebo group, overall and in the two subgroups. In the graphs for the ALSFRS-R score and slow vital capacity, I bars indicate 95% confidence intervals; in the handheld dynamometry megascore graphs, I bars indicate 1 SD.

in Table 3 and in Figures S6 through S9. Among the 10 participants who received tofersen in the 100-mg dose group, the least-squares mean ALSFRS-R score at day 85 changed from baseline by –1.19 points (95% CI, –4.67 to 2.29), as compared with a change of –5.63 points (95% CI, –8.90 to –2.36) among the 12 participants in the overall placebo group (Fig. 2). In the fast-progression subgroup, the ALSFRS-R score at day 85 changed from baseline by 0.84 points (95% CI, –5.58 to 7.26) in the group that received 100 mg of tofersen, as compared with –16.73 points (95% CI, –23.28 to –10.18) in the placebo group.

With regard to the percentage of the predicted slow vital capacity in the 100-mg tofersen group, the least-squares mean percent at day 85 changed from baseline by –7.08 percentage points (95% CI, –14.69 to 0.54), as compared with –14.46 percentage points (95% CI, –21.79 to –7.12) in the overall placebo group (Fig. 2). In the fast-progression subgroup, the percentage of the predicted slow vital capacity at day 85 changed from baseline by –8.62 percentage points (95% CI, –20.90 to 3.66) in the 100-mg dose group, as compared with –30.31 percentage points (95% CI, –43.28 to –17.34) in the placebo group.

The mean change from baseline in the handheld dynamometry megascore at day 92 was –0.03±0.18 in the 100-mg tofersen dose group, as compared with –0.26±0.42 in the overall placebo group (Fig. 2). In the fast-progression subgroup, the handheld dynamometry megascore changed from baseline at day 92 by 0.01±0.14 points in the 100-mg dose group, as compared with –0.52±0.42 points in the placebo group.

NEUROFILAMENTS

The baseline neurofilament concentrations were at least 3.5 times as high in the fast-progression subgroup as in the other subgroup. The concentrations of phosphorylated neurofilament heavy chains and neurofilament light chains in plasma and CSF were largely unchanged during the intervention period among the 12 participants in the placebo group at day 85, whereas among the 10 participants who received 100 mg of tofersen, the concentrations decreased from baseline to day 85. Data are shown in Figures S10 through S12. Changes in the plasma and CSF concentrations of phosphorylated neurofilament heavy chains and neurofilament light chains from day 85 to day 169 are shown in Figures S13 and S14.

DISCUSSION

In this trial of the SOD-1 mRNA-targeting ASO tofersen, adverse events were headache, procedural pain, post–lumbar puncture syndrome, falls, back pain, pain in an arm or leg, dizziness, and neck pain, many of which were attributable to the lumbar puncture that was required for administration of tofersen or placebo. Increased CSF protein and white-cell counts were also observed. The cause of CSF pleocytosis and protein elevations remains unclear. Although myelitis with sensory and motor deficits was not seen in this trial, the clinical syndrome has been observed in the context of tofersen administration (unpublished data from the ongoing phase 3 [part C] portion of our trial and the long-term extension study [ClinicalTrials.gov number, NCT03070119]). The underlying cause of myelitis and the rela-

tionship to CSF pleocytosis and protein elevations are unknown. Changes in CSF variables and any manifestations of central nervous system inflammation are being monitored in part C of our trial and in the ongoing extension study.

The reduction from baseline in the total CSF SOD1 concentration was 3% in the placebo group and 36% in the group that received 100 mg of tofersen (the highest-dose group), with reductions in the lower-dose tofersen groups ranging from 1% to 27%. There was no multiplicity adjustment of confidence intervals for the CSF SOD1 analyses, so no quantitative inferences can be made from these data. The highest concentrations of tofersen in plasma and CSF were observed with the 100-mg dose of tofersen. In post hoc analyses, there were no apparent differences in the baseline concentrations or magnitude of total reduction in the SOD1 concentration in CSF between participants with fast-progressing disease and other participants. In a finding that was possibly consistent with the half-life of SOD1 protein (approximately 30 days in humans),[20] the decrease in the SOD1 protein concentration was most apparent after 57 days. Data from animal models[9] suggest that ASOs can lower SOD1 protein concentrations by more than 75% in the spinal cord, but it is unknown how the reduction in the SOD1 concentration in CSF in the current trial translates into a reduction of SOD1 concentration in central nervous system parenchymal tissues.

This trial was not powered to test an effect on clinical or biologic measures beyond the reduction in SOD1 concentration in CSF. With regard to some exploratory outcomes, there may have been evidence of a slowing in the decrease in the ALSFRS-R score, the slow vital capacity, and the handheld dynamometry megascore with the 100-mg dose of tofersen, although no conclusions can be drawn from these descriptive outcomes. With cessation of the 100-mg dose of tofersen, smaller decreases in the SOD1 concentration in CSF, neurofilament light chains, and phosphorylated neurofilament heavy chains and greater decreases in the ALSFRS-R score were observed than during the intervention period.

Limitations of this early trial of tofersen in participants with ALS caused by *SOD1* mutations were the small number of participants, the short duration of treatment and follow-up, the exploratory nature of the efficacy outcomes, and the post hoc methods for defining the fast-progression subgroup as compared with the other subgroup. The safety and efficacy of tofersen are being evaluated in a phase 3, randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov number, NCT02623699) and its long-term extension study (NCT03070119).

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APPENDIX

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