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ORIGINAL ARTICLE

A randomized, double-blind, placebo-controlled phase 2 study to assess safety, tolerability, and efficacy of RT001 in patients with amyotrophic lateral sclerosis

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Abstract

Background and purpose: RT001 is a deuterated synthetic homologue of linoleic acid, which makes membrane polyunsaturated fatty acids resistant to lipid peroxidation, a process involved in motor neuron degeneration in amyotrophic lateral sclerosis (ALS).

Methods: We conducted a randomized, multicenter, placebo-controlled clinical trial. Patients with ALS were randomly allocated to receive either RT001 or placebo for 24 weeks. After the double-blind period, all patients received RT001 during an open-label phase for 24 weeks. The primary outcome measures were safety and tolerability. Key efficacy outcomes included the ALS Functional Rating Scale (ALSFRS-R), percent predicted slow vital capacity, and plasma neurofilament light chain concentration.

Results: In total, 43 patients (RT001 = 21; placebo = 22) were randomized. RT001 was well tolerated; one patient required dose reduction due to adverse events (AEs). Numerically, there were more AEs in the RT001 group compared to the placebo group (71% versus 55%, $p = 0.35$), with gastrointestinal symptoms being the most common (43% in RT001, 27% in placebo, $p = 0.35$). Two patients in the RT001 group experienced a serious AE, though unrelated to treatment. The least-squares mean difference in ALSFRS-R total score at week 24 of treatment was 1.90 (95% confidence interval = −1.39 to 5.19) in favor of RT001 ($p = 0.25$). The directions of other efficacy outcomes favored RT001 compared to placebo, although no inferential statistics were performed.

Conclusions: Initial data indicate that RT001 is safe and well tolerated. Given the exploratory nature of the study, a larger clinical trial is required to evaluate its efficacy.

KEYWORDS
amyotrophic lateral sclerosis, clinical trial, deuterated linoleic acid, lipid peroxidation, RT001

Leonard H. van den Berg and Ruben P. A. van Eijk share senior authorship.

INTRODUCTION

The pathophysiology underlying amyotrophic lateral sclerosis (ALS) is complex. Multiple unique disease pathways are associated with motor neurodegeneration and manifest with an ALS phenotype [1]. Oxidative stress and lipid peroxidation (LPO) are common pathophysiological elements involved not only in motor neurodegeneration, but also in glial and endothelial cell dysfunction [2, 3]. This has been well documented; significant increases in markers of LPO were observed in both preclinical models of ALS [4], and in patients with ALS [5]. Clinically, oxidative stress is believed to affect both the onset of the disease and the progression rate in patients [2], with edaravone, a free-radical scavenger to reduce oxidative stress, potentially slowing the progression rate in a subset of patients [6].

Given the strong association between oxidative stress and progression rate [7], antioxidative drugs could be a viable therapeutic strategy for ALS. RT001 is a deuterated synthetic homologue of linoleic acid (LA), substituting hydrogen with deuterium at bis-allylic sites, which makes membrane polyunsaturated fatty acids resistant to LPO. Use of deuterated polyunsaturated fatty acids, including LA, has decreased toxic products of LPO and improved mitochondrial function in multiple nonclinical models of oxidative stress [8–10]. Deuterated LA is not originally present in vivo.

Recently, in an expanded access program, RT001 was administered safely to 16 patients with ALS who were not eligible for other trials [11]. Adverse events were mild, and pharmacokinetic concentrations led to changes in membrane fatty acid composition, but the expanded access study did not include loading doses. Maintenance doses were also lower compared to those used in clinical trials for other neurodegenerative diseases such as Friedreich ataxia [12]. In this study, therefore, we investigated the safety, tolerability, and potential efficacy of a higher dose of RT001 in a randomized, placebo-controlled phase 2 trial in patients with ALS.

METHODS

Study design

This was a phase 2, randomized, double-blind, placebo-controlled study to test the safety, tolerability, and potential efficacy of RT001 in patients with ALS. Patients were recruited from four study sites in Estonia, Latvia, the Netherlands, and Sweden. All patients provided written informed consent. Patients were randomly assigned in a 1:1 ratio to receive either RT001 or a matching placebo. Randomization was stratified by study site. The duration of the study was 52 weeks, including a 4-week screening period and a 24-week double-blind treatment period. Patients who completed the 24-week treatment period could enter a 24-week open-label extension (OLE). The blind was maintained for all subjects and study personnel during the OLE. The trial was conducted in accordance with the provisions of the Declaration of Helsinki, the International Conference on Harmonization guidelines for Good Clinical Practice, and approved by the local ethics committees. The trial was registered under EudraCT number 2020–003962-38 and ClinicalTrials.gov number NCT04762589. The full study protocol is available in the Supplementary Material.

Study population

Eligible patients were aged between 20 and 75 years; they had an ALS diagnosis of probable laboratory-supported, probable, or definite ALS as defined by the El Escorial diagnostic criteria [13], an ALS Functional Rating Scale-Revised (ALSFRS-R) total score >20, were self-sufficient, meaning that they could eat a meal, excrete, and move around, and that they did not require caregiver assistance in such activities. Slow vital capacity (SVC) was ≥70% of the predicted value for age, height, ethnicity, and sex at screening, and symptom onset was <3 years prior to study entry.

Patients were excluded from the study if they had received other experimental therapies within 30 days prior to the first dose in this study; had previously received RT001; refused to discontinue the use of fish oils or other oil-based supplements for the duration of the study; had a feeding tube or the need for a feeding tube was anticipated during the study period; had a neurological disorder other than ALS; had a history of schizophrenia, schizoaffective disorder, or bipolar disorder according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition criteria or the International Classification of Diseases, tenth revision criteria; had a pulmonary disorder not attributed to ALS or required treatments that might complicate the evaluation of the effect of ALS on respiratory function; had a significant illness that required medical intervention in the last 30 days before screening; or if they had a history of alcohol abuse and/or physical opioid dependence. Genetic testing was not performed, and hence, both patients with familial and sporadic ALS were potential candidates for participation in the study.

Study medication

RT001 is an encapsulated 9-cis, 12-cis-11,11-D2-linoleic acid ethyl ester, which is a site-specific (C11) di-deutero synthetic homologue of linoleic acid ethyl ester. Each capsule contained 960 mg of RT001. The placebo product was composed of encapsulated United States Pharmacopeia safflower oil. The placebo capsules were identical in appearance, taste, and in size, and were indistinguishable from the study drug.

Randomized patients received nine capsules daily, given as three capsules thrice a day (TID) for the first 4 weeks of treatment. If the study drug was not tolerated, the dosage was reduced to six capsules (two capsules TID). After 4 weeks, the dose was reduced to six capsules daily, taken as three capsules twice a day for the remaining 20 weeks. If the study drug was not tolerated,

the dosing schedule could be changed to two capsules TID. If the study drug was still not tolerated, the dose was reduced to one to two capsules per day.

Outcome measures

Safety and tolerability were the primary outcome measures of the study and were evaluated by the incidence of serious adverse events (SAEs) and adverse events (AEs), and by the percentage of patients who were compliant with the drug regimen. Safety was evaluated at each visit by means of physical and neurological examination, testing of vital signs, electrocardiogram findings, and clinical laboratory test results to identify AEs. The safety of the study was continually reviewed by a study monitor. Adherence to the treatment was measured by comparing the number of capsules dispensed and returned.

The primary efficacy outcome was the change in the ALSFRS-R [14] total score from baseline to week 24 of treatment with assessments performed at screening, randomization, and weeks 8, 16, and 24. The secondary efficacy endpoints included the change from baseline in the ALS Assessment Questionnaire-40 (ALSAQ-40) [15], and change from baseline in percentage predicted SVC. In addition, we evaluated the composite endpoint time to death from any cause, tracheostomy, use of noninvasive ventilation, use of tube feeding, or increase in Milano-Torino stage (MiToS) [16]. Exploratory endpoints included the Clinical Global Impression of Severity (CGI-S), the plasma concentration of neurofilament light chain (NfL) protein, and muscle strength as measured with handheld dynamometry (HHD). The muscle groups assessed with HHD included elbow flexion, hand grip, and hip flexion.

To identify the pharmacokinetics of RT001, pharmacokinetic sampling was done at weeks 8 and 24 to determine the concentration of deuterated LA (D2-LA), deuterated arachidonic acid (AA) (D2-AA), nondeuterated LA (H2-LA), and nondeuterated AA (H2-AA) in plasma and red blood cells (RBCs). The blood samples were collected before breakfast and prior to the first daily dose of RT001. Measurements of D2-LA and H2-LA and AA were carried out using a validated high-performance liquid chromatography–tandem mass spectrometry method developed for the simultaneous determination of H2-AA, D2-AA, H2-LA, and D2-LA (RT001) in base hydrolyzed human blood plasma and red blood cells.

Sample size

The primary efficacy endpoint was the change in ALSFRS-R score from baseline to 24 weeks of treatment. The assumed treatment difference and standard deviation were derived from the analysis of the comparison of ALSFRS-R data of 16 patients receiving RT001 in an expanded access program [11]. The study was designed to have 83% power to detect a between-group difference of 3.96 ALSFRS-R points at week 24, with a common standard deviation of 4.20. For a two-sample *t*-test with a two-sided significance level of 0.05, a total of 40 patients (20 per group) was required.

Statistical analysis

Demographic data are presented using descriptive statistics (mean and standard deviation/median and interquartile range/frequency and percentage). For the analyses, the modified intention-to-treat (mITT) population consisted of all randomized patients who received at least one dose of RT001 or placebo and had at least one efficacy measure. The safety population included all patients who received at least one dose of RT001 or placebo.

SAEs and AEs were categorized by system organ class according to the standardized format of the Medical Dictionary of Regulatory Activities [17]. SAEs were reported as *n* (%), as number of times reported, and as a rate, with frequency of the AE being the numerator and total patient-years the denominator. For the assessment of treatment adherence, we report the percentage of patients who complied with the dosing schedule. Patients who took at least 90% of the prescribed dose were marked as compliant and considered to tolerate the full dosage. Differences in safety and treatment compliance between treatment groups were tested using Fisher's exact test. Safety and adherence analyses were performed in the safety population.

For the primary efficacy endpoint, the mean change in ALSFRS-R total score from baseline to 24 weeks of treatment, we fitted a mixed model for repeated measures (MMRM) using restricted maximum likelihood. The baseline ALSFRS-R total score, visit number, treatment-by-visit interaction, the study site, and the Treatment Initiative to Cure ALS (TRICALS) risk profile [18, 19]-by-visit interaction were included as fixed effects in the model. The TRICALS risk profile is a prognostic summary score of patient characteristics. Incorporating this variable in the model allowed us to account for several variables at once. An unstructured covariance structure was used to model the within-patient variances. Satterthwaite degrees of freedom were used to estimate the denominator degrees of freedom [20]. The least-squares mean difference between the treatment groups at week 24 was used for hypothesis testing using a two-sided significance level of 0.05. As a sensitivity analysis, the ALSFRS-R data were also analyzed using a linear mixed-effects (LME) model. The fixed part of the model was similar to the MMRM. A random intercept and a random slope for time for each patient were incorporated. Additionally, a leave-one-site-out analysis was performed for the ALSFRS-R data as a post hoc analysis, applying the MMRM model (using maximum likelihood) from the primary analysis, to rule out any site-specific effects. This was further explored by including a three-way interaction between visit, treatment, and study site, which was assessed using a likelihood ratio test (LRT).

The same analysis methods were used for the secondary and exploratory endpoints ALSAQ-40, percentage predicted SVC, muscle strength, CGI-S, and plasma NfL concentration. The composite

endpoint of time to death from any cause, tracheostomy, use of non-invasive ventilation, use of tube feeding, or an increase in MiToS was analyzed using Cox proportional hazards model with treatment and the risk profile as covariates.

All efficacy analyses were performed in the mITT population. Efficacy endpoints other than the primary endpoint were considered exploratory and presented solely with a point estimate and unadjusted 95% confidence intervals (CIs), and without inferential statements. Analyses were performed in R version 4.2.1 [21].

RESULTS

The trial was conducted between March 10, 2021 and August 30, 2022. In total, 49 patients were screened for eligibility, of whom 43 were included and randomly assigned to receive either RT001 ($n=21$) or placebo ($n=22$) (Figure 1). All 43 randomized patients were included in the mITT and safety analyses. In total, 91% of the randomized patients completed the 24-week randomized, double-blind treatment period (95% in the RT001 group, 86% in the placebo group). In total, three patients in the placebo group and one patient in the treatment group stopped the study prior to week 24, all due to withdrawal of their consent. The treatment groups were generally well balanced in demographic and clinical characteristics, although the patients in the RT001 group were older and had a numerically higher risk score, indicating a potentially poorer prognosis (Table 1).

Safety, tolerability, and treatment compliance

A summary of the SAEs is presented in Table 2. All AEs and SAEs were considered, regardless of causality between the event and the received treatment. There were no deaths during the 24-week treatment period. In the RT001 and placebo groups, 71% and 55%, respectively, experienced an AE ($p=0.35$). The AEs that occurred most frequently in both groups were gastrointestinal disorders, with a higher incidence in the RT001 group (43% compared to 27%, $p=0.35$). Three (14%) patients in the RT001 group experienced AEs that were severe in nature (pneumonia, fracture, loss of consciousness), but none of these severe AEs were deemed related to the study drug. One severe AE in the RT001 group led to early study discontinuation. Most of the AEs were mild to moderate (RT001 86%, placebo 100%, $p=0.26$).

Table S1 of the Supplementary Appendix presents the AEs during the OLE period. Based on a Poisson regression, the originally randomized placebo group had an AE rate of 1.90 (95% CI = 1.06–3.40) AEs per patient during the double-blind period, which increased to 2.04 (95% CI = 1.10–3.78) AEs per patient during the OLE period and a rate ratio of 1.07 (95% CI = 0.62–1.86).

Treatment compliance was adequate, with 17 (81%) of the patients in the RT001 arm and 14 (74%, 3 patients missing) in the placebo arm taking ≥90% of the study medication ($p=0.71$; Figure 2). For one patient in the RT001 group, the dosage of the drug was reduced due to an AE (gastrointestinal in nature) between weeks 8 and 16. For other noncompliant patients, a reduction in dosage was not prescribed. Overall, the patients in the RT001 group had better compliance with the treatment regimen.

Efficacy outcome measures

The estimated least-squares mean difference in ALSFRS-R total score between RT001 and placebo at week 24 of treatment was 1.90 points (95% CI = −1.39 to 5.19) in favor of RT001 ($p=0.25$) (Table 3). Figure 3 shows the course of the ALSFRS-R change from baseline least-squares means per treatment group over time, including the OLE period. The mean difference at week 56 (end of OLE) was 4.10 points (95% CI = −1.76 to 9.96). The LME model provided similar results, with a mean monthly rate of decline of −0.54 points per month (95% CI = −0.93 to −0.15) for the RT001 group and of −0.81 points per month (95% CI = −1.20 to −0.43) for the placebo group during the 24-week placebo-controlled period, resulting in a mean slope difference of 0.27 points per month (95% CI = −0.28 to 0.82), or a 33% reduction. There was no statistically significant interaction between the treatment effect, visit, and site ($p_{LRT}>0.99$). The leave-one-site-out sensitivity analyses for the ALSFRS-R are available in the Supplementary Appendix (Figure S1). The full primary model output is presented in the Supplementary Appendix (Table S2). Of note, the TRICALS risk profile-by-visit interaction was a strong predictor ($p_{LRT}<0.01$), meaning that patients with a poorer prognosis have a greater decline in the ALSFRS-R score.

The results for the secondary and exploratory endpoints are presented in Table 3. Due to the explorative nature of the secondary efficacy analyses, no p-values are given. However, the direction of the effect estimates on the time-to-event endpoint, ALSAQ-40, SVC, muscle strength (except hand grip), and NfL are in line with the primary efficacy endpoint.

Pharmacokinetics

Overall, 21 patients in the RT001 arm underwent pharmacokinetic sampling at weeks 8 and 24 of treatment; for 2 patients, the RBC measurements at week 8 are not available, and for 1 patient, the measurements (both plasma and RBC) at week 24 are not available. The ratio of plasma D2-LA to LA was 18.26 (±5.17%) at week 8 and 20.44 (±7.05%) at week 24 of treatment. The ratio of plasma D2-AA to AA was 11.05 (±3.35%) at week 8 and 14.25 (±4.52%) at week 24. For the RBC samples, the ratio of D2-LA to LA was 19.09 (±5.99%) at week 8 and 20.40 (±5.25%) at week 24. Finally, the ratio of RBC D2-AA to AA was 9.28 (±3.01%) at week 8 and 13.62 (±3.32%) at week 24. Additional information pertaining to the association between pharmacokinetics and the ALSFRS-R can be found in the Supplementary Appendix (Figure S2). No clear correlation was observed between the magnitude of increase in deuterated fatty acids and the ALSFRS-R.

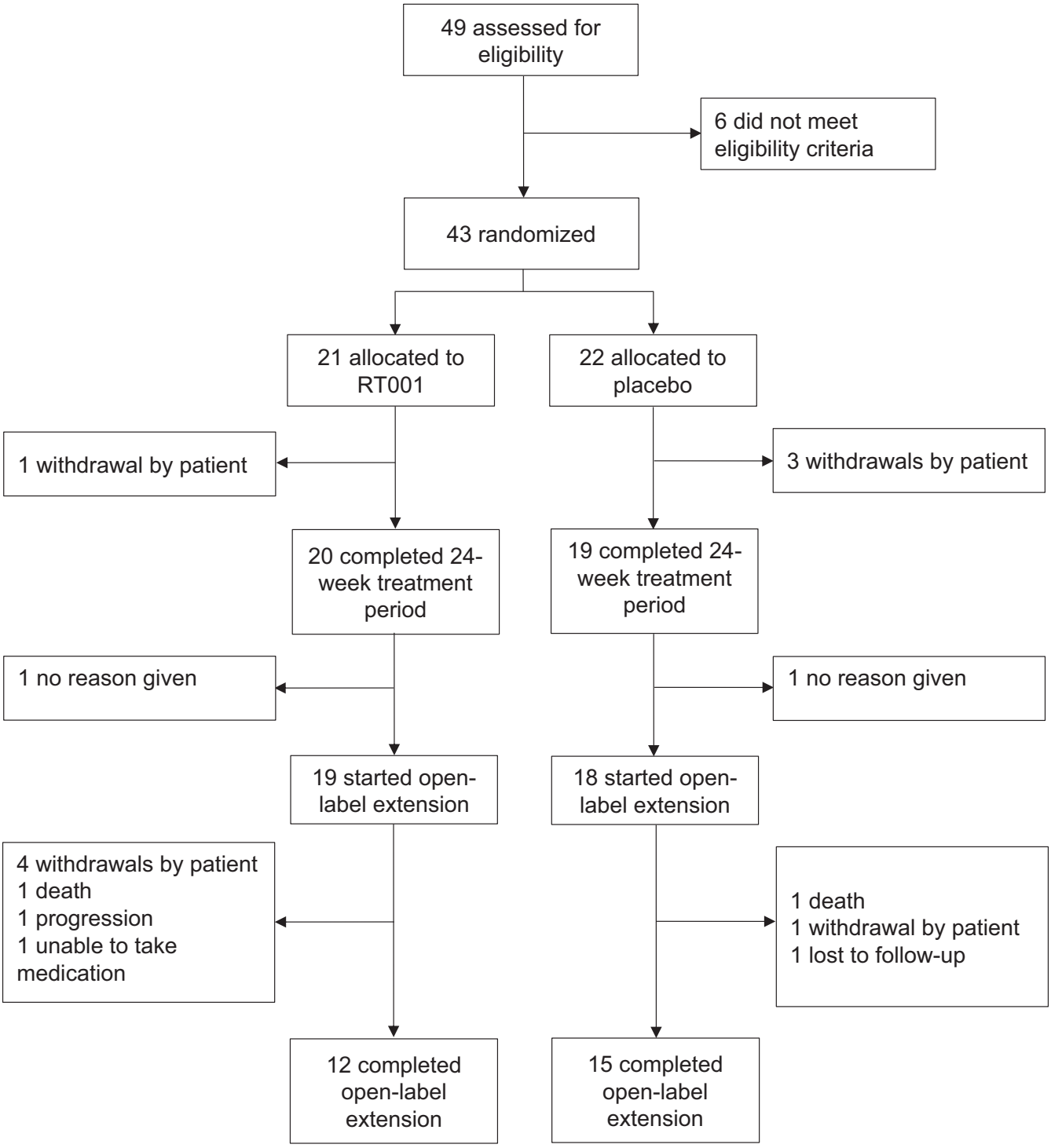

FIGURE 1 Study flow diagram. All 21 patients allocated to the RT001 arm and all 22 patients allocated to the placebo arm received at least one dose of the drug therapy/placebo. Additionally, all 21 RT001 patients and all 22 placebo patients were used in the safety and modified intention-to-treat analyses.

DISCUSSION

This phase 2 study assessed the safety, tolerability, and early efficacy of RT001 over a period of 24 weeks in patients with ALS. The results show that RT001 is safe and well tolerated. None of the SAEs were judged to be related to the treatment and occurred similarly in the placebo arm. Gastrointestinal AEs occurred numerically more frequently in the RT001 group, though were mild to moderate in nature and self-limiting. The study was primarily exploratory in nature and not powered for small to moderate clinical effects; nevertheless, the directional effects across different clinical efficacy endpoints are consistent and favor RT001. A larger and longer study is needed

TABLE 1 Baseline characteristics.

Characteristic	RT001 (*n* = 21)	Placebo (*n* = 22)	All patients (*n* = 43)
Age, years	62.9 (8.3)	58.7 (9.2)	60.7 (8.9)
Sex, male	10 (48%)	13 (59%)	23 (53%)
Site of onset, bulbar	3 (14%)	6 (27%)	9 (21%)
Symptom duration, months[a]	16.9 (12.6)	15.6 (5.7)	15.9 (10.7)
ALSFRS-R score at baseline	38.6 (4.1)	38.0 (5.6)	38.3 (4.9)
ΔFRS, points/month[a]	−0.48 (0.45)	−0.47 (0.40)	−0.48 (0.44)
% Predicted upright SVC at baseline	90.2 (14.0)	91.1 (16.5)	90.7 (15.2)
TRICALS risk score[b]	−4.25 (1.12)	−4.69 (1.19)	−4.48 (1.16)
BMI, kg/m^2	25.5 (4.5)	24.8 (3.1)	25.1 (3.8)
Concomitant riluzole use, yes	14 (67%)	15 (68%)	29 (67%)

Note: Data are *n* (%) or mean (SD). Maximum possible score for the ALSFRS-R is 48.

Abbreviations: ΔFRS, baseline ALSFRS-R score - 48 divided by the symptom duration; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; BMI, body mass index; SVC, slow vital capacity; TRICALS, Treatment Initiative to Cure Amyotrophic Lateral Sclerosis.

[a]Median (interquartile range).

[b]The range of the TRICALS risk score varies approximately between −12 to 0, with higher scores (less negative) indicating a poorer prognosis.

TABLE 2 Adverse events and serious adverse events during the 24-week placebo-controlled treatment period.

	RT001 (*n* = 21)			Placebo (*n* = 22)		
Variable	*n* (%)	Total frequency	Rate	*n* (%)	Total frequency	Rate
Any adverse event	15 (71%)	42	3.74	12 (55%)	28	2.54
Specific adverse events						
Gastrointestinal disorders	9 (43%)	13	1.16	6 (27%)	9	0.82
General disorders and administration site conditions	3 (14%)	3	0.27	3 (14%)	4	0.36
Infections and infestations	1 (5%)	1	0.09	1 (5%)	1	0.09
Injury, poisoning, and procedural complications	6 (29%)	13	1.16	4 (18%)	7	0.63
Musculoskeletal and connective tissue disorders	2 (10%)	3	0.18	2 (9%)	3	0.27
Nervous system disorders	3 (14%)	3	0.27	3 (14%)	4	0.36
Psychiatric disorders	1 (5%)	1	0.09	—	—	—
Renal and urinary disorders	1 (5%)	1	0.09	—	—	—
Reproductive system and breast disorders	1 (5%)	1	0.09	—	—	—
Skin and subcutaneous tissue disorders	1 (5%)	1	0.09	—	—	—
Vascular disorders	2 (10%)	2	0.18	—	—	—
Severity of adverse events						
Mild	13 (62%)	32	2.85	12 (55%)	26	2.36
Moderate	5 (24%)	7	0.62	2 (9%)	2	0.18
Severe	3 (14%)	3	0.27	—	—	—
Serious adverse events	2 (10%)	2	0.18	1 (5%)	1	0.09
Adverse event leading to discontinuation of study	1 (5%)	1	0.09	—	—	—
Deaths	—	—	—	—	—	—

Note: The rate was calculated as the total frequency of adverse events divided by the summed follow-up time across all patients within that treatment group. The follow-up time is 11.23 person-years in the RT001 group and 11.03 person-years in the placebo group.

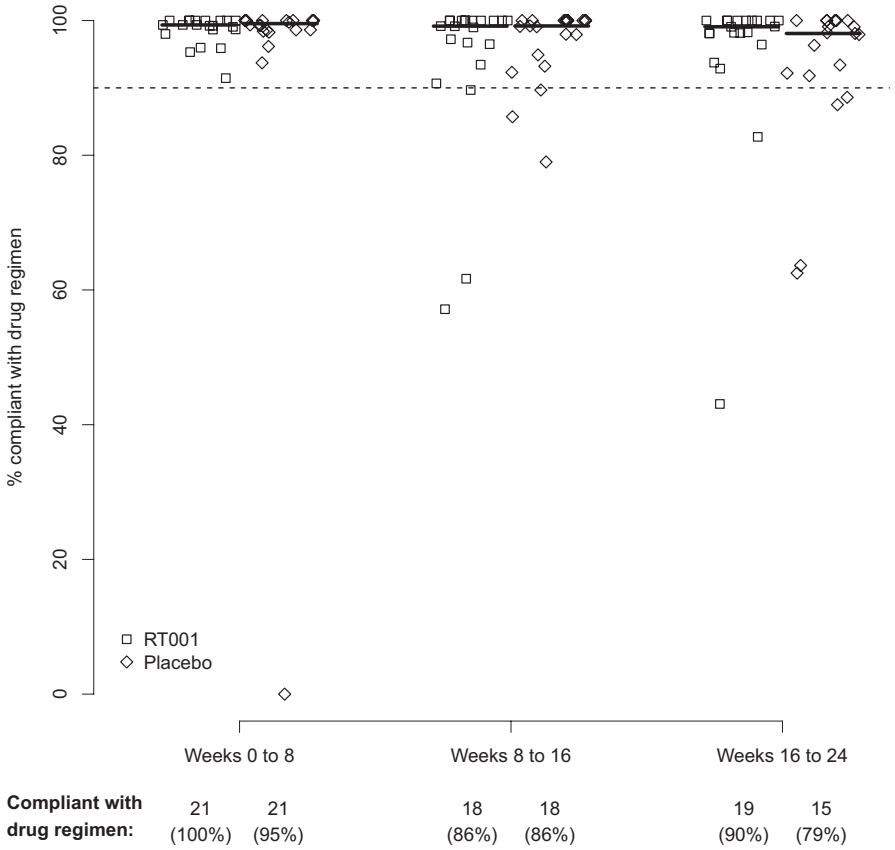

FIGURE 2 Adherence to the administration regimen during the double-blind treatment period. The horizontal lines mark the median compliance per visit per treatment group. The dashed line marks the 90% cutoff point for being compliant (≥90%) or noncompliant (<90%). The numbers on the bottom indicate the absolute number (percentage of total between parentheses) of patients who were compliant with the drug/placebo regimen. Patients with zero compliance returned the exact amount/more of capsules than were dispensed. One placebo patient (between 0 and 8 weeks) dropped out of the study and was 0% compliant.

TABLE 3 Summary of results for the primary, secondary, and exploratory efficacy endpoints at week 24 of the treatment period.

	RT001 ($n=21$)	Placebo ($n=22$)	Mean difference	p
Primary endpoint				
ALSFRS-R, MMRM	−3.00 (−5.31 to −0.70)	−4.91 (−7.23 to −2.58)	1.90 (−1.39 to 5.19)	0.25
ALSFRS-R, LME	−0.54 (−0.93 to −0.15)	−0.81 (−1.20 to −0.43)	0.27 (−0.28 to 0.82)	—
Secondary endpoint: time-to-event data				
Composite-free survival[a]	50.0% (30.4 to 82.4)	39.0% (19.7 to 77.2)	HR 0.57 (0.21 to 1.51)	—
Secondary endpoints: continuous data				
ALSAQ-40	14.20 (7.05 to 21.35)	18.86 (11.63 to 26.08)	−4.66 (−14.67 to 5.35)	—
SVC (%)	−5.50 (−11.80 to 0.80)	−9.37 (−15.58 to −3.16)	3.87 (−5.00 to 12.73)	—
CGI-S	3.81 (3.52 to 4.11)	3.70 (3.37 to 4.04)	0.11 (−0.29 to 0.51)	—
HHD (*N*)				
Elbow flexion	70.20 (52.62 to 87.78)	65.35 (48.07 to 82.62)	4.85 (−18.68 to 28.39)	—
Hand grip	29.60 (22.43 to 36.77)	32.79 (25.68 to 39.90)	−3.19 (−12.83 to 6.45)	—
Hip flexion	79.83 (54.15 to 105.50)	77.11 (51.63 to 102.59)	2.71 (−31.77 to 37.20)	—
Plasma NfL concentration[b]	55.57 (38.93 to 79.32)	59.83 (41.86 to 85.51)	RR 0.93 (0.58 to 1.50)	—

Note: Results are estimate (95% confidence interval) for both groups. ALSFRS-R, ALSAQ-40, and SVC are presented as change from baseline through 24 weeks of follow-up.

Abbreviations: ALSAQ-40, Amyotrophic Lateral Sclerosis Assessment Questionnaire-40; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; CGI-S, Clinical Global Impression of Severity; HHD, handheld dynamometry; HR, hazard ratio; LME, linear mixed-effects model; MMRM, mixed model for repeated measures; NfL, neurofilament light chain; RR, risk ratio; SVC, slow vital capacity.

[a]Death/specified stage of disease progression is expressed as a 24-week event-free probability (per treatment group, in percentages) and hazard ratio.

[b]The plasma NfL concentration is expressed as geometric mean (per treatment group) and geometric mean ratio (i.e., RR).

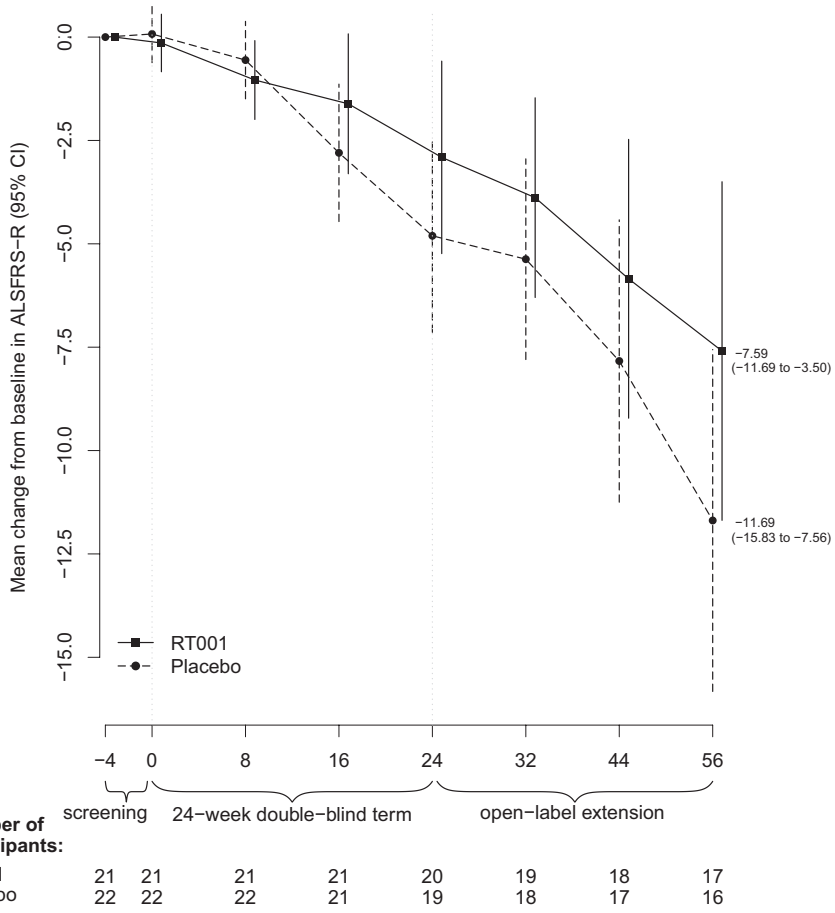

FIGURE 3 Mean change from baseline in Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) total score between groups during the placebo-controlled treatment period and the open-label extension. Mixed model for repeated measures analysis providing the least square means at each visit for each treatment arm. The double-blind period lasted from week 0 to 24, after which all patients were offered active treatment (open-label extension). The numbers on the right of the figure mark the least-squared means per treatment group (95% confidence interval [CI] in parentheses) at the end of the open-label extension. The numbers at the bottom indicate the number of patients still in the trial per point in time per treatment group.

to further validate the results of this study and to formally assess the efficacy of RT001 in ALS.

RT001 was recently provided during an expanded access program in the United States, which found that RT001 can be safely administered to patients with ALS [11]. The program, however, used a lower dose compared to our study (starting dose of 2.88 g/day compared to 8.64 g/day used in this study). As such, our study additionally demonstrates the safety of higher doses of RT001 in patients with ALS. Significantly, an increased ratio of plasma D2-LA to LA was achieved after 24 weeks of treatment, increasing from 14.1% observed previously to 20.4% (±7.1%). Previous research in model systems has shown that the inhibition of LPO occurs when the membrane concentration of D2-LA reaches a level of about 10%, and plateaus around 20% [8]. In the current study, RT001 was absorbed, elongated into D2-AA, and incorporated in RBC membranes at levels that have been reported to attenuate LPO [8].

During the study, we noted a higher incidence of gastrointestinal AEs, which may be related to RT001. Nevertheless, a similar incidence of gastrointestinal events was observed among placebo-treated patients; these did not seem to increase when patients were switched to active treatment during the OLE period. It should be noted, however, that the placebo treatment consisted of dietary safflower oil that is enriched in LA. Hence, it is possible that the use of dietary oils in general led to an increased incidence of gastrointestinal events.

Considering the primary objective of our study was to assess safety and tolerability, the major strengths of the study design were the direct control by a randomized placebo arm and the continuation of the study into a blinded OLE period. The randomization of patients, which reduces the influence of (unobserved) confounding factors, amplified the safety signal during the placebo-controlled period, and helped to put AE rates into perspective. Moreover, by including the OLE period, we could study a change in AE rates in patients originally randomized to placebo. Finally, the OLE period allowed for an extended monitoring period in the patients originally randomized to active treatment, providing helpful information on long-term safety and tolerability for the design of a future pivotal study.

The OLE period can be viewed as a delayed-start design and can help to further evaluate efficacy signals. For example, one might test whether patients originally randomized to placebo catch up with patients originally randomized to RT001. If this is the case, such an effect would be suggestive of a temporary, symptomatic effect of RT001 rather than a true disease-modifying effect [22, 23]. In our study, the mean differences in clinical efficacy endpoints remained relatively stable during the OLE period, which would be in line with a disease-modifying effect. Significantly, there may be presence of continued divergence between treatment arms after all patients are switched to active treatment. This may suggest that the group who started treatment early may experience a larger benefit of treatment compared to the group who started treatment later. Such an effect

would align with the hypothesis that treatment effects may be largest when treatment is initiated very early in the disease, as has been suggested previously [24]. Nevertheless, our sample size was too limited to formally test the change in between-group differences over time and, as such, no inferential statements could be made. Making better use of OLE efficacy information, however, for example by prospectively defining such disease-modifying hypotheses, could be an important objective for future clinical trials. These types of analyses might be further improved by refining the set of covariates that improve statistical power. For example, we incorporated the TRICALS risk profile given its strong association with the patient's progression rate [19], thereby explaining part of the variability between patients and increasing the precision of the study [25].

In conclusion, in this study we have shown that treatment of ALS patients with high-dose RT001 for up to 54 weeks is feasible and tolerable. Two patients in the RT001 group experienced an SAE, though unrelated to treatment. Overall, AE rates were comparable between RT001 and placebo, and were primarily self-limiting in nature. Clinical efficacy endpoints favored RT001 compared to placebo, but given the exploratory nature of the study, a larger clinical trial is required to evaluate its efficacy.

AUTHOR CONTRIBUTIONS

Daphne N. Weemering: Conceptualization, formal analysis, visualization, writing–draft preparation, writing–review and editing. **Mark Midei:** Conceptualization, writing–review and editing. **Peter Milner:** Conceptualization, writing–review and editing. **Vidhya Gopalakrishnan:** Conceptualization, writing–review and editing. **Anil Kumar:** Conceptualization, writing–review and editing. **Andrew J. Dannenberg:** Conceptualization, writing–review and editing. **Tommy M. Bunte:** Investigation, writing–review and editing. **Juliette Foucher:** Investigation, writing–review and editing. **Caroline Ingre:** Investigation, writing–review and editing. **Viktorija Ķēniņa:** Investigation, writing–review and editing. **Karin Rallmann:** Investigation, writing–review and editing. **Leonard H. van den Berg:** Conceptualization, investigation, supervision, writing–review and editing. **Ruben P. A. van Eijk:** Conceptualization, formal analysis, supervision, writing–draft preparation, writing–review and editing.

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CONFLICT OF INTEREST STATEMENT

D.N.W., T.M.B., J.F., C.I., V.Ķ., K.R., L.H.v.d.B., and R.P.A.v.E. declare no competing interests for this work. M.M., P.M., V.G., and A.K. are employees of BioJiva. A.J.D. has served as a consultant to BioJiva.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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