STATISTICAL ANALYSIS PLAN

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1 TABLE OF CONTENTS

2 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Non-abbreviated term
ALS	Amyotrophic Lateral Sclerosis
ALSAQ-40	ALS assessment questionnaire-40
ALSFRS-R	ALS Functional Rating Scale-Revised
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
Al-P	Alkaline Phosphatase
AUC	Area Under the Blood Concentration-Time Curve from Zero to Infinity
bipap	Bilevel (biphasic) Positive Airway Pressure mask
BUN	Blood Urea Nitrogen
CK	Creatine Kinase (creatine phosphokinase)
CMAP	Compound Muscle Action Potential
C_{max}	Maximum Plasma Concentration
CL	Total Clearance
DCF	Data Clarification Form
FAS	Full Analysis Set
GAD	Gracile Axonal Dystrophy
GCP	Good Clinical Practice
γ-GTP	γ-Glutamyl Transpeptidase
HERG	Human Ether-a-go-go Related Gene
IVH	Intravenous Hyperalimentation
LDH	Lactate Dehydrogenase
MMT	Manual Muscle Testing
MRC score	Medical Research Council score
NAMDRC	National Association for Medical Direction of Respiratory Care
NMDA	N-Methyl-D-Aspartate
NV	Non-Invasive Ventilation
PEG	Percutaneous Endoscopic Gastrostomy
PPS	Per Protocol Set
PT	Preferred Term
QT	QT interval
QTcF	QT interval corrected for heart rate using Fridericia's formula
SOC	System Organ Class
SOD1	Superoxide Dismutase
SpO_2	Saturation of Hemoglobin with Oxygen using Pulse Oximetry
$t_{1/2}$	terminal half-life
t_{max}	time at maximum observed concentration
Vss	Volume of Distribution at Steady State
WFN	World Federation of Neurology

3 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the procedures and statistical methods to be used for analyzing the protocol <E0302-J081-761> and reporting the statistical results.

3.1 STUDY OBJECTIVES

3.1.1 Primary Objective(s)

To evaluate the dose response and verify the superiority over placebo by conducting a multi-center, randomized, parallel-group, double-blind, comparative study in patients with amyotrophic lateral sclerosis (ALS), regarding the time to onset of events due to E0302 (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) and the ALS Functional Rating Scale (ALSFRS-R).

3.1.2 Secondary Objective(s)

Not set in this study.

3.1.3 Exploratory Objective(s)

Not set in this study.

3.2 OVERALL STUDY DESIGN AND PLAN

This study is a multi-center, randomized, placebo-controlled, parallel-group, double-blind, comparative study in patients with ALS.

The investigator determines the eligibility at the time of start of the observation period of patients who have given consent to participate in the study in writing, and register them in the 12-week observation period (see "Protocol 9.4.2.1 Procedures for Patient Registration and Study Drug Assignment"). After the 12-week observation period, the investigator determines the eligibility at the time of completion of the observation period.

The registration center dynamically assigns subjects, who are confirmed eligible at the time of completion of the observation period, to the placebo group, E0302 25 mg group, or E0302 50 mg group for the treatment period (see "Protocol 9.6.7 Method of Assignment). The study drugs are delivered within 2 weeks from the completion of the observation period.

The primary endpoints of the study are the time to onset of events (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) and the amount of change in the total ALSFRS-R score from the time of completion of the observation period.

The treatment period is 182 weeks. However, the treatment will be completed when an event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) occurs.

In order to secure the blindness of the study as much as possible, the study drug treatment, efficacy evaluation, safety evaluation, and ALSFRS-R evaluation are each conducted by a separate independent person at a study institution as a general rule. A person in charge of the efficacy evaluation, a person in charge of the ALSFR-R evaluation, and a person in charge of the safety

evaluation conduct evaluation necessary at Weeks 4, 16, 28, 40, 52, 64, 76, 88, 100, 112, 124, 136, 148, 160, 172 and 182, and at the onset of an event or at the time of discontinuation.

Of note, the safety was evaluated as the 1st interim analysis based on the data up to 1 year after the registration for the treatment period was started (The 1st Meeting of Independent Data Monitoring Committee: August 5, 2008; the study was recommended to be "continued" on the same day). The 2nd interim analysis was to be conducted to examine the safety and efficacy based on the data up to the occurrence of the 80th event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death). However, because 80 events did not occur by March 2010, the 2nd interim analysis was conducted based on the data up to the end of March 2010 (The 2nd Meeting of Independent Data Monitoring Committee: October 27, 2010; the study was recommended to be "continued" on the same day).

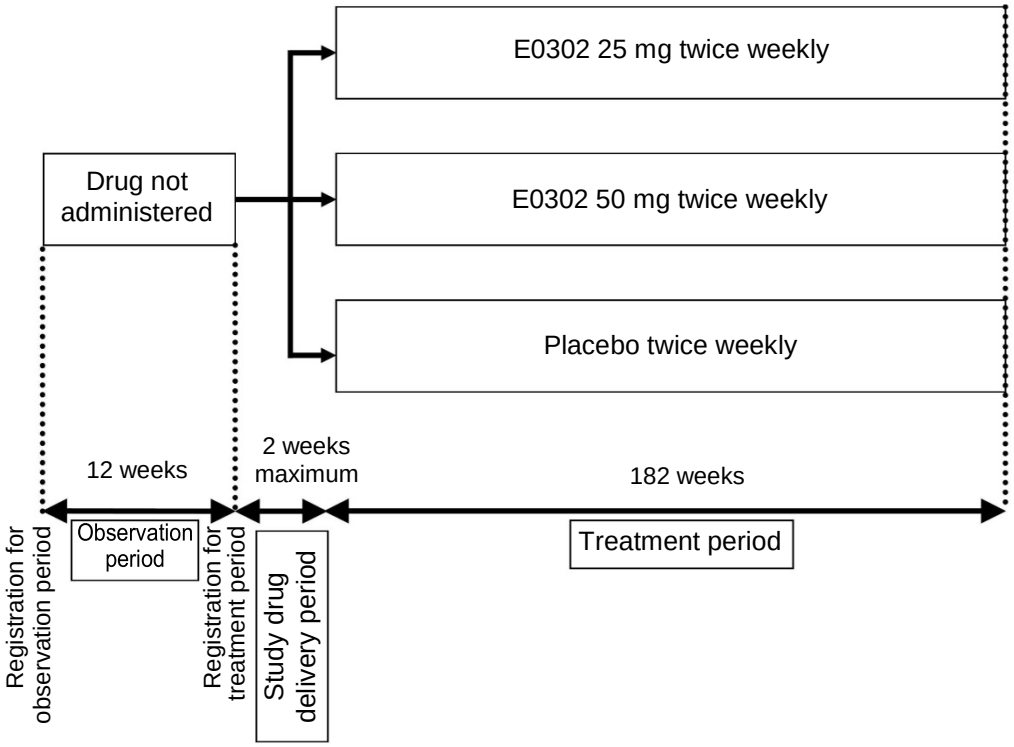

Figure: Study Flow

4 DETERMINATION OF SAMPLE SIZE

The sample size for the study was initially 100 for each group, or 300 in total with 3 groups. However, it was changed to 120 for each group, or 360 in total with 3 groups based on the results of the blind data monitoring during the study. The basis for the sample size and the course of change are described below.

(1) Method of analysis

The study is a randomized comparative study in 3 groups (placebo, E0302 25 mg, and E0302 50 mg) with two primary endpoints (time to full-time wearing of non-invasive ventilation, wearing of

invasive ventilation, or death, and the amount of change in the total ALSFRS-R score from pretreatment). Based on either of the two primary endpoints, the dose response and the superiority of E0302 over placebo are examined by adjusting the multiplicity.

To adjust the multiplicity, the following four contrasts are set to calculate the adjustment p-value by sorting to examine and interpret based on the significance. If p-value from the contrast [-1, 0, 1] is smaller than the contrast [-2, 1, 1], the dose response is flat, and if opposite, the dose response saturates. The logrank score is used for the amount of statistic for contrasting for the time to full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death. The Wilcoxon score is used for the amount of change in the total ALSFRS-R score from pretreatment.

- [Placebo, 25 mg, 50 mg] = Logrank score for [–1,0,1]
  (Time to event is extended in a dose-dependent manner)
- [Placebo, 25 mg, 50 mg] = Logrank score for [–2,1,1]
  (Time to event peaks out in the 25 mg group)
- [Placebo, 25 mg, 50 mg] = Wilcoxon score for [–1,0,1]
  (The reduction of ALSFRS-R is minimized in a dose-dependent manner)
- [Placebo, 25 mg, 50 mg] = Wilcoxon score for [–2,1,1]
  (The reduction of ALSFRS-R peaks out in the 25 mg group)

The adjustment p-value is calculated according to the following steps, and the contrast below 2.5% on one-sided is considered significant.

- Calculate p-values (one-sided) for the four contrasts, and arrange them in ascending order.
- Rearrange them using random numbers, and calculate the sorting distribution for the smallest, the 2nd smallest, the 3rd smallest and the largest of the p-value.
- Use the p-value for each contrast calculated from the empirical distribution above as the adjustment p-value, and contrasts with the adjustment p-value below 2.5% one-sided in the ascending order of the p-values are regarded as verified contrasts.

The multitest procedure is used for data rearrangement to create the empirical distribution. One hundred thousand sets of rearranged data are generated with the subject number, the number of dates until event onset and censoring, the presence/absence of event/discontinuation/completion, the amount of change at the end in total ALSFRS-R score and the ranked score as 1 set of observation. Four empirical distributions are generated for the p-value for each contrast calculated from each rearranged data.

(2) Endpoints used for calculation of sample size and estimates

Full-time wearing of non-invasive ventilation, wearing of invasive ventilation, and death are regarded as events, and the incidence of these events in the long-term administration study of mecobalamin in ALS patients was estimated. As a result, the incidence of events at Month 24 in the mecobalamin 50 mg group and the non-treatment group was 0.45 and 0.75, respectively (hazard ratio: 0.431). However, it needs to be considered that this study was an open-label, non-randomized, controlled study, the criterion for events were different, and there was variation due to the small number of the subjects in the study. Therefore, it is assumed that the annual incidence of events in the

entire study was 0.50~0.55 with the hazard ratio of 0.5~0.6. On the other hand, there is no report examining the transition of ALSFRS-R with long-term administration of mecobalamin. No statistical significance was observed or no drug was demonstrated to be effective in any of the previously conducted placebo controlled studies with ALSFRS-R as the primary endpoint. Therefore, it needs to use the criteria based on the ratio of the difference between groups Δ and the standard deviation within group σ in order to calculate the sample size with expected clinical effects with ALSFRS-R. In the QOL data analysis, 0.3~0.4, which is often called significant, is to be used.

(3) Calculation of sample size

In the analysis of the two primary efficacy endpoints, the four contrasts are tested by considering the multiplicity. Therefore, its influence on the number of subjects and the power is evaluated from simulation. With the non-centrality corresponding to the difference between groups as $\Delta = n^{1/2}\Delta/\sigma$, it is assumed that both E0302 25 mg and E0302 50 mg are superior to the placebo group by the non-centrality Δ. The power with which the contrast [-2, 1, 1] is significant without adjusting the multiplicity is as follows.

Δ	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Power	6.0	12.6	23.1	37.2	53.2	68.8	81.5	90.4	95.7

The results of simulation show that when the four contrasts are tested simultaneously by considering the multiplicity, the power of 80% or higher can also be secured if Δ of one of the variables is 4.0 or higher or if one of Δ is 3.5 or higher and the other is 2.5 or higher. In such cases, the change in the boundary based on the coefficient of correlation ρ were small, and even with any values of coefficient of correlation ρ, the boundary was approximately around 2.35. The boundary 2.35 is equivalent to the significance level of 1% on one-sided.

When comparing with preset 1 variable and 2 groups (active drug 1 dose and placebo), Δ with the power 80% is 3.96. That is to say, in order to examine 2 variables and 2 doses, sacrifices of need to be made that the number of subjects is increased by approximately 1.5 fold and the power is increased by 10%. The t-test on continuous quantity (Wilcoxon test), normal approximation test on logarithmic hazard ratio for survival time (mostly corresponds to logrank test), required Δ (3.5~4) and Δ/σ or hazard ratio (active drug/placebo), and required number of subjects per group and events are calculated as follows.

Required number of subjects per group (or number of events)

		n	
δ/σ hazard ratio (exp ($-\delta/\sigma$))		Δ = 3.5	4.0
0.2	0.82	307	400
0.3	0.74	137	178
0.4	0.67	77	100
0.5	0.60	49	64
0.6	0.55	34	45

When $\Delta=4$, the number of events per group that corresponds to hazard ratio 0.60 is 64, and it is 190 for the 3 groups. The number of subjects per group that corresponds to Δ/σ =0.4~0.5 is 64~100, respectively.

On the other hand, the total number of events that guarantees 90% power with 1% on one-sided test by the logrank test between the 3 groups (contrast [-2, 1, 1]) is approximately 200 using the Freedman's formula, which is roughly consistent with the value above.

Accordingly, at least 80 subjects is necessary for each group, and a total of 200 events is necessary. By taking into consideration discontinuation of the study in some subjects, the sample size for the study is determined to be 100 each group, or 300 in total.

In accordance with the provisions of "Protocol 12.2.3.2. Data Monitoring," data monitoring was conducted in subjects who received the study drug between March 26, 2007 (the date when the 1st subject was registered for the treatment period) to December 31, 2008. The results confirmed that the annual incidence of events was 0.178, which was lower than the annual incidence of events predicted at the time of planning (0.50-0.55). If this annual incidence of events continues to be maintained, it would be difficult to secure 200 events at the end even if the treatment period is extended by 1 year (the treatment period to 3 years and 6 months). Therefore, the required number of events that guarantees 80% power with 1% on one-sided test by the logrank test between the 3 groups (contrast [-2, 1, 1]) using the Freedman's formula was changed to 153.

Additional data monitoring was conducted in subjects who received the study drug between March 26, 2007 (the date when the 1st subject was registered for the treatment period) to May 31, 2009. The results showed that the annual incidence of event was 0.192 and the annual rate of discontinuation was 0.131. Based on these results, the number of subjects required to secure the target number of events at the time of final analysis, which was 153, was estimated by simulation, and the results showed that a total of 355 subjects need to be registered for the treatment period. Therefore, the number of sample size was changed to 360 (120 subjects for each group) by also considering that the test is a 3-group comparative study.

5 STATISTICAL METHODS

The significance level for the test is 15% on two-sided as the standard to determine the consistency between groups, 2.5% on one-sided as the standard for significance for efficacy endpoints, and 5% on two-sided for others.

If the person conducting analysis determines the analysis plan needs changes or additions after the study is started, the person responsible for analysis and the person in charge of the study examine the appropriateness of such changes or additions and their impact on the study evaluation and determine whether the changes or additions to be made. Important changes to the analysis plan and the results of the analysis are described in the study report.

Summary statistics for continuous variables are presented as the average, standard deviation (SD), median, minimum, and maximum unless otherwise noted. Classification variables are summarized by the number of subjects and percentage (%).

5.1 STUDY ENDPOINTS

5.1.1 Primary Endpoint(s)

(1) The time to onset of event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) from registration for the treatment period

(2) The amount of change in the total ALSFRS-R score from pretreatment

5.1.2 Secondary Endpoint(s)

(1) MMT
(2) Norris scale
(3) %FVC
(4) Grip strength
(5) ALSAQ-40
(6) SpO_2 during sleep
(7) Symptoms and signs
(8) Laboratory test values
(9) ECG
(10) Vital signs

5.1.3 Exploratory Endpoint(s)

Not applicable.

5.1.4 Pharmacokinetic Endpoints

Not applicable.

5.2 STUDY SUBJECTS

5.2.1 Definitions of Analysis Sets

There are two analysis sets for the efficacy: “Full analysis set” and “Per Protocol Set.” However, the primary analysis set is the full analysis set, and the per protocol set is regarded as a secondary analysis set to confirm the consistency with the results obtained from the full analysis set from a perspective of sensitive analysis.

Full Analysis Set (FAS) is a set of subjects registered for the treatment period excluding those who:

- do not meet the primary inclusion criteria (inclusion criteria 1~3)
- received treatment outside the contracted period, or violated GCP such as no consent
- have no data regarding the primary endpoints (time to full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death, ALSFRS-R) that can be evaluated
- did not receive treatment

Per Protocol Set (PPS) is a set of subjects registered for the treatment period excluding those who:

- do not meet the primary inclusion criteria (inclusion criteria 1~3)

- received treatment outside the contracted period, or violated GCP such as not giving consent
- have no data after Week 52 of study drug treatment regarding the primary endpoints (time to full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death, ALSFRS-R) that can be evaluated and did not experience the events
- meet the exclusion criteria that may influence the efficacy evaluation (exclusion criteria 1~7, 14)
- have less than 70% cumulative rate of study drug injection for up to the date of completion or discontinuation
- changed the daily dose of riluzole before Week 52
- did not receive treatment

Data are excluded if the case conference determines it may influence the efficacy evaluation. **Safety Analyses Set** is a set of subjects registered for the treatment period excluding those who:

- have not given consent
- registered while the study institution and the sponsor have not concluded the study contract
- do not meet the inclusion criteria concerning the main target diseases (inclusion criteria 1~3)
- did not receive treatment
- have no safety data that can be evaluated

5.2.2 Subject Disposition

The numbers of subjects registered for the treatment period who received treatment and who did not receive treatment are calculated. The numbers and the ratios of subjects received the treatment who completed the study and who discontinued the study were calculated by treatment group and in total. As for the subjects who completed the study, the numbers and the ratios are calculated for each event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) and for study period completion by treatment group and in total. As for the subjects who discontinued the study, the numbers and the ratios for each reason are calculated by treatment group and in total. Similarly, the numbers of subjects registered for the treatment period who were included in and excluded from each analysis set and the ratios are calculated by treatment group and in total.

A list of breakdown of the subjects and the reasons for discontinuation is created.

A list of the subject identification codes and the groups assigned to is created.

5.2.3 Protocol Deviations

The definitions of the breakdown of the subjects of the study are shown in the table below.

Table Definitions of the breakdown of the subjects

Term	Definition
Subjects registered for the observation period	Subjects who were considered eligible at the start of the observation period by the investigator, and confirmed and registered by the registration center for the observation period.
Subjects discontinued the observation period	Subjects who registered for the observation period and discontinued the study before being registered for the treatment period.
Subjects registered for the treatment period	Subjects who were considered eligible at the end of the observation period by the investigator, and confirmed and registered by the registration center for the treatment period.
Subjects who did not	Subjects registered for the treatment period who did not receive the study drug.

receive treatment	
Subjects who completed the treatment	· Subjects who completed the treatment period: Subjects who completed the treatment period · Subjects who completed the study due to an event: Subjects who experienced an event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) and completed the study (including those who were assessed to have experienced no event by the case conference)
Subjects who discontinued the study	Subjects who discontinued the study before completion of the treatment period because the study discontinuation criterion was met after the study drug treatment started.

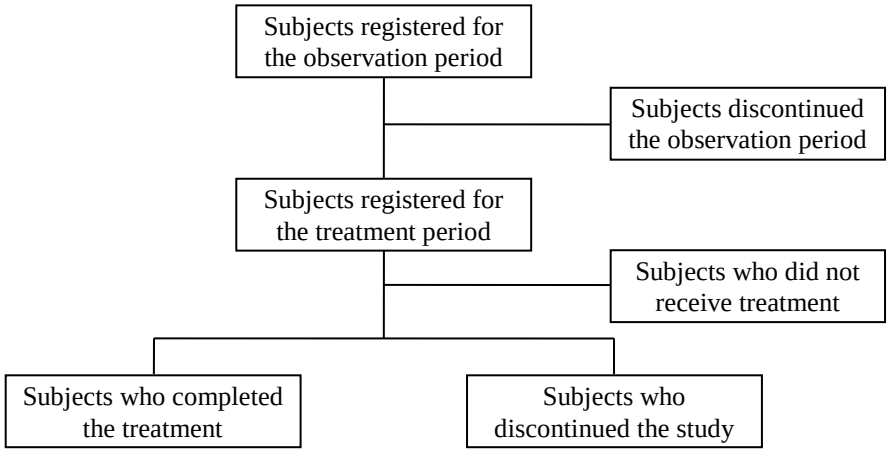

Figure The breakdown of the subjects

5.2.4 Demographic and Other Baseline Characteristics

- The following summary statistics for the continuous variables are calculated for the endpoints concerning demographic and pretreatment characteristics of each analysis set (safety analysis set, FAS, and PPS) by treatment group and in total. Similarly, the numbers of subjects for each classification and the ratios for the following classification variables for the endpoints concerning demographic and pretreatment characteristics as well as the continuous variables classified according to the summary segment as needed are calculated by treatment group and in total. The consistency between groups are confirmed by one-way analysis of variance for the continuous variables, by chi-square test for the continuous variables classified according to the summary segment and the classification variables, and by Kruskal-Wallis test for the ordinal classification variables.

Timing for obtaining data	Continuous variables	Classification variables
At the start of observation period	Age, ALSFRS-R, %FVC	Age (<65 years/≥65 years), sex, initial symptoms (bulbar onset, arm onset, leg onset), ALS onset type (sporadic, familial)
At the end of observation period	ALSFRS-R, time from ALS onset until the start of treatment period, %FVC, amount of change in %FVC during the observation period	Use of riluzole during the observation period, revised version of El Escorial and Airlie House Diagnostic Criteria (clinically definite ALS / clinically probable ALS / clinically-probable-laboratory-supported ALS), severity criteria for ALS at the end of observation period (Degree 1/Degree 2), change in the total ALSFRS-R score during the observation period

(-1 point/-2 points/-3 points)

- A list of demographic data based on all date acquired is created.

5.2.5 Medical History

- A list of medical history and complications is created. Verbatim terms for medical history and complications listed on the case report form are converted using Medical Dictionary for Regulatory Activities (MedDRA) Ver. 16.1, and the verbatim terms as well as converted system organ class (SOC) and preferred term (PT) of MedDRA are used.

5.2.6 Prior and Concomitant Therapy

- A list of pretreatment drugs/concomitant drugs is created. Verbatim terms for pretreatment drugs and concomitant drugs listed on the case report form are coded using Iyakuhinmei Data File by MT Kyogikai and its terms are used.

5.2.7 Treatment Compliance

- The summary statistics for the number of injection and the cumulative rate of injection up to the study completion or discontinuation are calculated for FAS. Statistics are gathered by treatment group and for total. The number of subjects included in each summary segment and the ratio are calculated (summary segment: <70%/ 70-100%/ >100%). The applicable period (weeks) and the cumulative rate of injection are defined by the following formulas.

  Applicable period (weeks) = (Final date of prescribed injection – Start date of E0302 injection + 1) /7
  Note: The digits after the decimal point, obtained from the formula for the applicable period (weeks), are rounded down to a whole number. The final date of prescribed injection is the onset date of event for subjects who experienced events and the date of discontinuation for subjects who discontinued the study (subjects who experienced no event after discontinuation), and the applicable period (weeks) is 1274 (182 weeks × 7) for subjects who completed the treatment period.

  The number of injection (times) = (the number of days with "○" for the treatment status for up to the final date of prescribed injection)

  Cumulative rate of injection (%) = The number of injection (times) × 100 / [applicable period (weeks) × 2]

- A list of the medication status is created.

5.3 DATA ANALYSIS GENERAL CONSIDERATIONS

5.3.1 Pooling of Centers

The sample size for the study is 360 (120 for each group). Since it is determined that the number of subjects per study institution is too small to evaluate the interaction between study treatment and study institution, no analysis which takes study institutions into account will be conducted.

5.3.2 Adjustments for Covariates

Of all background factors examined in the FAS described in Section 5.2.4 as patients characteristics that may influence the clinical evaluation of ALS, the disease under the study, the influence on the primary endpoints by the background items for which the consistency between treatment groups could not be confirmed is examined using Cox regression for the time to the onset of event, and Tobit model with data given the lowest rank (see 8 DEFFINITIONS AND CONVENTIONS FOR DATA HANDLING) as truncated data for the amount of change in the total ALSFRS-R score at the end. No other analysis adjusted by covariates will be conducted. The LIFEREG procedure is used for Tobit model specifying "NORMAL" distribution type for the option of the MODEL statement. The minimum value for subjects excluding those with no ALSFRS-R score after death or onset of event is specified as the objective variable. Even if analysis adjusted for imbalances is conducted, the results obtained from analysis not adjusted for imbalances are regarded as the main analysis results.

5.3.3 Multiple Comparisons/Multiplicity

Analysis is conducted by taking into account the multiplicity. The details are described in Section 4 and Section 5.4.1

5.3.4 Examination of Subgroups

Analysis similar to the one described in Section 5.4.1.1 is conducted on the amount of change in the total ALSFRS-R score from the registration for the treatment period to the onset of event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) as well as from the end of observation period to the end of treatment by the following subgroup in the FAS. However, the multiplicity is not considered in the tests, and the p-value from a test using the logrank score for the period up to the onset of event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) is calculated, and the p-value from a test for contrast using the Wilcoxon score for the amount of change in the total ALSFRS-R score from the end of observation period to the end of the treatment is calculated.

<Subgroups>

Age (<65 years old/≥65 years old), sex, initial symptoms (bulbar onset/extremity onset [arm onset, leg onset]), ALS onset type (sporadic, familial), time from the onset of ALS to the start of treatment (≥18 months /<18 months), %FVC (≥90%/<90%), amount of change in %FVC (≥0/<0) during the observation period, use of riluzole during the observation period, revised version of El Escorial and Airlie House Diagnostic Criteria (clinically definite ALS / clinically probable ALS / clinically-probable-laboratory-supported ALS) at the end of the observation period, severity criteria for ALS at the end of observation period (Degree 1/Degree 2), change in the total ALSFRS-R score during the observation period (-1 point/-2 points/-3 points)

5.3.5 Handling of Missing Data, Drop-outs, and Outliers

Specified in "8 DEFFINITIONS AND CONVENTIONS FOR DATA HANDLING."

5.3.6 Other Considerations

None.

5.4 EFFICACY ANALYSES

The significance level for the test is 15% on two-sided as the standard to determine the consistency between groups, 2.5% on one-sided as the standard for significance for efficacy endpoints, and 5% on two-sided for others.

Calculation of estimates in analysis other than the time to event analysis is performed for 2 types of cases as a set: a case when all subjects in each analysis set are targeted and a case when only subjects whose final data has been evaluated in each analysis set are targeted (ie, a set of subjects excluding those whose data after death or event onset has not been measured).

5.4.1 Analysis of Primary endpoint

5.4.1.1 Primary Analysis

Analysis item

- The time to onset of events (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death)
- The amount of change in the total ALSFRS-R score from the time of completion of the observation period to the end of the treatment.

Analysis set

FAS (primary analysis), PPS

Analysis method

The logrank score is calculated for the time to onset of events, and the Wilcoxon score is calculated for ALSFRS-R. Based on statistics obtained, one-sided p-value is calculated for each contrast shown in Section 4, and the adjustment p-value is calculated by rearranging. For reference, each p-value which does not take the multiplicity into account is calculated as well.

The table below defines event/censoring for each subject in the primary analysis. Events/censoring defined below are summarized.

Table Definitions of censoring and event

Situation	End Date	Event / Censoring
Subject died	Onset date of event (date of death)	Event
Full-time wearing of non-invasive ventilation	Onset date of event (date of full-time wearing of non-invasive ventilation)	Event
Wearing of non-invasive ventilation	Onset date of event (date of wearing of non-invasive ventilation)	Event
Subject who discontinued the study and did not experience the above events within 28 days after the date of final dosing	Date of discontinuation	Censoring
Treatment completed	Week 180, which is the lower limit of acceptable period for evaluation at Week 182 after treatment (Day 1261)	Censoring

As summary statistics for each group, 50% event occurrence period, 25% event occurrence period, 75% event occurrence period, and 95% confidence intervals for each of them, as well as hazard ratios for the 25 mg group and the 50 mg group to the placebo group and their 95% confidence intervals are calculated. Similarly, the average hazard ratio and 95% confidence interval for contrast [-2, 1, 1].

As sensitivity analysis for the primary analysis, the above analysis is conducted based on the definitions of event/censoring for each subject as described below.

Table Definitions of censoring and event (sensitive analysis)

Situation	End Date	Event / Censoring
Subject died	Onset date of event (date of death)	Event
Full-time wearing of non-invasive ventilation	Onset date of event (date of full-time wearing of non-invasive ventilation)	Event
Wearing of non-invasive ventilation	Onset date of event (date of wearing of non-invasive ventilation)	Event
Subject discontinued (subjects who experienced an event after discontinuation of the study are regarded as "subjects who discontinued the study")	Date of discontinuation	Censoring
Subject completed treatment	Week 180, which is the lower limit of acceptable period for evaluation at Week 182 after treatment (Day 1261)	Censoring

Summary statistics (median, 25% point, 75% point, minimum, maximum) are calculated by group for the amount of change at the end of treatment in the total ALSFRS-R score to the ALSFRS-score at the end of the observation period. The average value, the standard deviation, and 95% confidence interval are also noted for reference. The method of calculating the Wilcoxon scores is specified in "8 DEFFINITIONS AND CONVENTIONS FOR DATA HANDLING."

The amount of change in the total ALSFRS-R score at the end of treatment
= ALSFRS-R at the end of treatment – ALSFRS-R at the completion of the observation period

5.4.1.2 Secondary Analysis

Analysis item

- The time from registration for the treatment period to onset of events (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death)
- The amount of change in the total ALSFRS-R score from the time of completion of the observation period.
- The time from registration to the treatment period to onset of each event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death)

Analysis set

FAS (primary analysis), PPS

Analysis method

The analysis specified below is summarized by treatment group. Summary statistics for events (cumulative rate of occurrence of event and 95% confidence interval) are calculated at Weeks 28, 52,

76, 100, 124, 148, 172, and 182. ALSFRS-R is evaluated at the start of observation period, the completion of observation period, Weeks 4, 16, 28, 40, 52, 64, 76, 88, 100, 112, 124, 136, 148, 160, 172, and 182, and the end of treatment. The amount of change is a difference from the completion of observation period.

For the period up to the event occurrence, the survival curves are calculated using the Kaplan-Meier method for each group and for 2 groups: the placebo group and the active drug group (25 mg group and 50 mg group combined). The logrank test is conducted for the period from Week 52 to the event occurrence as well as the survival curve is presented in a diagram for each group using the Kaplan-Meier method (landmark analysis beginning at Week 52). When presenting the diagram, at-risk subjects are calculated by group for each evaluation time point during the treatment period. Similarly, the cumulative rate of event occurrence and 95% confidence interval are also calculated for each evaluation time point (Week 52 after registration for the treatment period and thereafter). Summary statistics (median, 25% point, 75% point, minimum, maximum) are calculated by group for the actually measured value and the amount of change in the total ALSFRS-R score at each evaluation time point. The average value and the standard deviation are also noted for reference. Contrast of the primary analysis is tested for each of them. The change in the median of the amount of change in ALSFRS-R is presented in a diagram with the amount of change on the vertical axis and the time point on the horizontal axis.

The slope of the change speed in ALSFRS-R is calculated by fitting the straight line to the successive change pattern of ALSFRS-R for each subject. Summary statistics (average, standard deviation, median, 25% point, 75% point, minimum, maximum) are calculated by group for this slope. The slope is calculated by simple linear regression analysis with actual values of ALSFRS-R obtained successively from each subject as the objective variable, and the number of days from the start of treatment to the day of each measurement as the explanatory variable. The slope is compared between groups by t-test of contrast [-1, 0, 1] and contrast [-2, 1, 1].

Similarly, summary statistics (median, 25% point, 75% point, minimum, maximum) are also calculated for the amount of change in the actual value and the pretreatment value on the day of each evaluation specified for 3 categories of scores of ALFRS-R (total score of bulbar function “speech,” “salivation,” and “swallowing”; limb function: “handwriting,” “eating habits,” “dressing and hygiene,” “turning in bed,” “walking,” and “climbing stairs”; respiratory function: “dyspnea,” “orthopnea,” and “respiratory insufficiency). The average value and the standard deviation are also noted as reference. The Wilcoxon’s rank sum test is conducted on contrast [-1, 0, 1] and contrast [-2, 1, 1] for the 3 categories of scores.

5.4.2 Analysis of Secondary Endpoint

Analysis item

- Total MMT score, score for each site measured
- Norris scale
- %FVC
- Grip strength
- ALSAQ-40
- SpO_2 during sleep

Analysis set

FAS (primary analysis), PPS

Analysis method

The analysis specified below is summarized by treatment group. The time points for evaluation are the same as ALSFRS-R described in Section 5.4.1.2. The amount of change is a difference from the completion of observation period.

Summary statistics (median, 25% point, 75% point, minimum, maximum) are calculated by group for the measured values at each evaluation time point and the amount of change from the time of completion of the observation period to each evaluation time point in the total ALSFRS-R score for the total MMT score and score for each site measured, Norris scale (total score, total Limb Norris Scale, total Norris Bulbar Scale), %FVC, grip strength (for left and right obtained on each evaluation day), and ALSAQ-40 (total, Physical Mobility [10 items: 1-10], ADL/Independence [10 items: 11-20], Eating and Drinking [3 items: 21-23], Communication [7 items: 24-30], and Emotional Functioning [10 items: 31-40]). The average value and the standard deviation are also note as reference. The Wilcoxon score of subjects whose data was not obtained after an event was observed is calculated by interpolating with the subject's worst value of the amount of change.

For the total MMT score, total Norris scale score, %FVC, grip strength (left and right), and the amount of change in the total ALSAQ-40 score at the end of the treatment period, the Wilcoxon's score and p-value for each of the following contrasts are calculated.

[Placebo group, 25 mg, 50 mg] = Wilcoxon's score for [-1, 0, 1]
(reduction in measured value can be minimized in a dose-dependent manner)

[Placebo group, 25 mg, 50 mg] = Wilcoxon's score for [-2, 1, 1]
(reduction in measured value peaks out in the 25 mg group)

Summary statistics of SpO_2 during sleep is calculated by group for each evaluation time point. The evaluation is conducted during the observation period, every 4 weeks (Week 4 – Week 176 after treatment) beginning on the start date of treatment, Week 182 after treatment, and the end of treatment.

<Lists>

Lists are created for efficacy data (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) and the time to onset of these events, ALSFRS-R, MMT score and score for each site measured, Norris scale (total score, total Limb Norris Scale, total Norris Bulbar Scale), %FVC, grip strength (left and right), ALSAQ-40 (total, Physical Mobility (10 items: 1-10), ADL/Independence (10 items: 11-20), Eating and Drinking (3 items: 21-23), Communication (7 items: 24-30), Emotional Functioning (10 items: 31-40), representative value of SpO_2 during sleep).

5.5 PHARMACOKINETIC/PHARMACODYNAMIC/PHARMACOGENOMIC ANALYSES

Not applicable.

5.6 SAFETY ANALYSES

The safety analysis is conducted on the safety analysis set. The safety endpoints include adverse events, laboratory test values, and vital signs.

5.6.1 Extent of Exposure

The endpoint regarding the exposure status is defined as follows.

Duration of exposure (days) = [Last date of treatment] – [Start date of study drug treatment] + 1

Summary statistics are calculated by treatment group for duration of exposure. As categorical analysis, the number and the ratio of patients with duration of exposure of more than 52 weeks (1 year), more than 104 weeks (2 years), and more than 156 weeks (3 years) are calculated. Additionally, a list of the exposure status is created.

5.6.2 Adverse Events

Analysis items: Adverse events/adverse reactions by patient and by item (SOC, PT), serious adverse events, adverse events leading to discontinuation

Analysis set: Safety analysis set

Analysis method:

The analysis specified below are conducted by treatment group and for the total of E0302 groups.

Summary by subject

The number of cases and the incidence are calculated for adverse events/adverse reactions, severe adverse events/adverse reactions, serious adverse events/adverse reactions, death, other serious adverse events/adverse reactions, breakdown of serious adverse events/adverse reactions, and adverse events/adverse reactions leading to discontinuation. The 95% confidence interval (F distribution) is calculated for the incidence of adverse events/adverse reactions, and the placebo group and each active drug group are compared using Fisher's exact test.

Summary by item (SOC, PT)

The number of cases and the incidence are calculated for adverse events/adverse reactions. They are also summarized by severity. Adverse events are additionally summarized by causality.

The number of cases of serious adverse events is summarized, and the incidence of serious adverse events, and its 95% confidence interval (F distribution) are calculated. The incidence of serious adverse events is compared between the placebo group and each active drug group using Fisher's exact test. Similarly, analysis is also conducted on serious adverse events excluding "death" due to worsening of symptoms associated with progression of primary disease.

The number of cases of adverse events leading to discontinuation of the study drug (excluding death) is summarized, and the incidence of adverse events leading to discontinuation of the study drug and its 95% confidence interval (F distribution) are calculated.

The number of cases and the incidence are calculated for each subgroup defined below. The classification criteria for seriousness of adverse reactions due to pharmaceutical products (criteria for abnormal changes in laboratory test results) used in Appendix 7 of the protocol should be referred to for the grades of hepatic dysfunction and renal dysfunction.

<Subgroups>
Age (<65 years old/≥65 years old), sex, initial symptoms (bulbar onset/extremity onset [arm onset, leg onset]), ALS onset type (sporadic, familial), time from the onset of ALS to the start of treatment (≥18 months/<18 months), %FVC (≥90%/<90%) at the end of the observation period, amount of change in %FVC (≥0/<0) during the observation period (≥median of all subjects/<median), use of riluzole during the observation period, revised version of El Escorial and Airlie House Diagnostic Criteria (clinically definite ALS / clinically probable ALS / clinically-probable-laboratory-supported ALS) at the end of the observation period, severity criteria for ALS at the end of observation period (Degree 1/Degree 2), change in the total ALSFRS-R score during the observation period (-1 point/-2 points/-3 points), by severity of hepatic dysfunction at screening (normal, Grade 1, Grade 2), by severity of renal dysfunction at screening (normal, Grade 1, Grade 2)

The number of cases of adverse events/adverse reactions are summarized by period of initial occurrence (by every 12 weeks, at Week 180 and thereafter). Only the number of subjects who experienced adverse events/reactions is compiled. Additionally, summary statistics (the number of cases, median, minimum, maximum) for the number of days until initial occurrence of adverse event are calculated by item (SOC, PT).

An event that was observed multiple times in the same patient is categorized into “events associated most frequently with the drug” (“probably related” > “possible related” > “unrelated”) to be summarized for the number of cases by causality. It is categorized into “severe events” (“severe” > “moderate” > “mild”) to be summarized for the number of cases by severity.

<Lists>
- A list of deaths, a list of serious adverse events, and a list of adverse events leading to discontinuation are created.
- A list of adverse events by subject is created.

5.6.3 Laboratory Values

Analysis item : Abnormal changes by subject and by item, laboratory test values at each evaluation time point
Analysis set : Safety analysis set
Analysis method :

The analysis specified below is summarized by treatment group. The time points for evaluation are the same as ALSFRS-R described in Section 5.4.1.2. The amount of change is a difference from the completion of observation period.

- The incidence of abnormal change is calculated, and the placebo group and each active drug group are compared using Fisher’s exact test. Data of abnormal changes obtained from case report forms are used.
- Summary statistics for measured values of and the amount of change in each item of hematology and serum chemistry tests are calculated by time of evaluation. For urinalysis, a cross tabulation of the number of cases and the ratio (%) by time of evaluation to those before the start of treatment is created.
- The amount of change in each item of hematology and serum chemistry tests at each evaluation time point is compared with group using 1-sample Wilcoxon test. For urinalysis, the change at each

evaluation time point before the start of treatment is presented as either “no change,” “aggravated,” or “improved” and compared within group using 1-sample Wilcoxon test. It is also compared between the placebo group and each active drug group using 2-sample Wilcoxon test.
- A diagram of the temporal change in values of each item of hematology and serum chemistry tests is created using box-plot.

<List>
- A list of laboratory test values by subject is created.

5.6.4 Vital Signs

Analysis item : Vital signs (blood pressure [systolic/diastolic]) at each evaluation time point, abnormal change by item (blood pressure, pulse, ECG)
Analysis set : Safety analysis set
Analysis method :

The analysis specified below is summarized by treatment group. The time points for evaluation are the same as ALSFRS-R described in Section 5.4.1.2. The amount of change is a difference from the completion of observation period.
- The incidence of abnormal change is calculated, and the placebo group and each active drug group are compared using Fisher’s exact test. Data of abnormal changes obtained from case report forms are used.
- Summary statistics for values measured at each evaluation time point and the amount of change are calculated.
- The amount of change at each evaluation time point is compared within group using 1-sample Wilcoxon test. It is also compared between the placebo group and each active drug group using 2-sample Wilcoxon test.
- A diagram of the temporal change in values measured is created using box-plot.
- The number of cases and the ratio of subjects in whom abnormal change was observed in one of the evaluation time points after the start of treatment (including tests at non-scheduled visit and tests at discontinuation, and excluding follow-up examination) and the number of subjects with missing data are calculated.
- A cross tabulation of the presence of abnormality of ECG at each evaluation time point to the presence of abnormality at the end of the observation period is created, and the placebo group and each active drug group are compared using Fisher’s exact test. They are also compared using McNemar test.

<List>
- A list of vital signs by subject is created.

5.6.5 Other Safety Analyses

No safety analysis is conducted other than those described above.

5.7 OTHER ANALYSES

Not applicable.

5.8 EXPLORATORY ANALYSES

If exploratory analysis is conducted and the results of exploratory analysis is reported in the clinical study report, it should be done in a way so that the analysis can be clearly distinguished from other analysis specified in the analysis plan.

5.9 EXTENSION PHASE ANALYSES

Not applicable.

6 INTERIM ANALYSES

In this study, interim analysis was conducted twice. In the 1st interim analysis, the safety analysis was conducted based on the data up to 1 year after the registration for the treatment period was started (The 1st Meeting of Independent Data Monitoring Committee: August 5, 2008; the study was recommended to be "continued" on the same day). The 2nd interim analysis was to be conducted to examine the safety and efficacy based on the data up to the occurrence of the 80th event (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death). However, because 80 events did not occur by March 2010, the 2nd interim analysis was to be conducted based on the data up to the end of March 2010 (The 2nd Meeting of Independent Data Monitoring Committee: October 27, 2010; the study was recommended to be "continued" on the same day). Both interim analyses were conducted an open condition according to "Procedure for Independent Data Monitoring," "Operating Procedure for Interim Analysis," and "Interim Analysis Plan (1st interim analysis and 2nd interim analysis)."

6.1 INTERIM ANALYSIS 1ST

The 1st interim analysis evaluates the interim safety. It is conducted by the Independent Data Monitoring Committee openly based on the data up to 1 year after the registration for the treatment period was started. The interim safety analysis summarizes serious adverse events by treatment group, adverse events leading to discontinuation, adverse events by type and severity, adverse drug reactions, and discontinuation to examine whether the study can be continued from a safety standpoint. Based on the results of the safety interim analysis, the Independent Data Monitoring Committee recommends the sponsor to either "discontinue the study due to safety reasons" or "continue the study." As a result of the 1st Meeting of the Independent Data Monitoring Committee held on August 5, 2008, the study was recommended to be continued on the same day.

6.2 INTERIM ANALYSIS 2ND

The 2nd interim analysis evaluates the efficacy and safety. The total 153 events (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) are required to verify the efficacy in this study. The 2nd interim analysis is to be conducted after 80 events (approximately a half of 153 events) have occurred, by the Independent Data Monitoring Committee under an open condition. However, if 80 events do not occur by March 2010, the 2nd interim analysis is to be conducted based on the data up to the end of March 2010. The Independent Data Monitoring Committee is to comprehensively evaluate the interim efficacy and safety results of the 2nd interim analysis, and recommends the sponsor to either "discontinue the study due to inefficacy" or "continue the study." As a result of the 2nd Meeting of Independent Data Monitoring Committee held on October 27, 2010, the study was recommended to be "continued" on the same day.

6.2.1 EFFICACY EVALUATION

The interim analysis of the efficacy evaluates the time to onset of events (full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death) and the amount of change in the total

ALSFRS-R score. If observation is continued for up to 153 events of full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death, the Bayes predictive probability is calculated for each event when two contrasts by the logrank test exceeds 2.5% on one-sided. If the amount of change is continued for up to Week 182 (3 years and 6 months), the Bayes predictive probability is calculated when two contrasts of the Wilcoxon scores of ALSFRS-R exceeds 2.5% on one-sided. Based on the two kinds of predictive probability calculated, the study is determined to be "discontinued due to inefficacy" or "continued."

1) Discontinuation due to inefficacy
   In the following cases, the efficacy of E0302 cannot be validated, and the study is recommended to be discontinued due to inefficacy (futility). The limit is 0.22 in the standard normal distribution, or an unadjusted p-value of 0.41.
   Predictive probability by the logrank test : all below 5%
   Predictive probability by the Wilcoxon score : all below 5%

2) Continuation
   In cases other than the above "discontinuation due to inefficacy," the study is recommended to be "continued." If the study is to be continued, the average period of treatment of all subjects, from which the study's required number of events of 153 can be obtained, is calculated based on the status of occurrence of each event of full-time wearing of non-invasive ventilation, wearing of invasive ventilation, or death in each treatment group. Based on the calculated period of treatment, a recommendation is given to the sponsor revise the protocol regarding shortening or extending the period of treatment. However, even if the period is to be shortened, the treatment period of each subject must be 2 years or longer. The period can be extended up to 3 years and 6 months, and if the extension is recommended to be longer than that, the study will be discontinued.

6.2.2 SAFETY EVALUATION

The interim analysis of safety summarizes serious adverse events by treatment group, adverse events leading to discontinuation, adverse events by type/severity, adverse drug reactions, and discontinuation to examine whether the study can be continued from a safety standpoint.

6.3 RECOMMENDATION FROM INDEPENDENT DATA MONITORING COMMITTEE

The following recommendations are given to the sponsor by the Independent Data Monitoring Committee based on the results of the 1st and 2nd interim analysis.

1st interim analysis: discontinue due to safety reasons, continue

2nd interim analysis: discontinue due to inefficacy, discontinue due to safety reasons, continue (including the treatment period)

The review process and reasons for the recommendation are not to be disclosed until the final analysis is unblinded. However, this does not apply to the case where the study is recommended to be discontinued due to safety reasons and the Independent Data Monitoring Committee considers that there is urgency in respect to securing the safety of subjects.

7 CHANGES IN THE PLANNED ANALYSES

The following changes, additions, and major corrections were made to the statistical methods (Protocol 12. Method of Analysis) specified in the protocol, ver. 10 (created on June 25, 2010).

- It was planned to examine the influence on the primary endpoints using multiple regression analysis in adjusted analysis with covariates for the amount of change in ALSFRS-R. However, for subjects who died or with no data after event occurrence, their worst value is assigned in the Wilcoxon test, and it is therefore appropriate to handle the amount of change at the end of these subjects as truncated data. Accordingly, the method was changed to confirm the influence of bias of background factors by conducting adjusted analysis with Tobit model, with which the lowest value of the continuous quantity can be set to be used as censored data.

- Regarding summary of adverse events/adverse reactions, summary of the number of cases was deleted with the intent to output data in accordance with presentation of the number of cases of adverse events described in "CTD format related to application for shortening of total evaluation period for new drug (Notice, January 17, 2011)."

- The cumulative incidence of adverse events/adverse reactions occurring at high frequency was to be calculated by group using Kaplan-Meier method. However, analysis of particular adverse events was deleted since occurrence of adverse reaction under a blinded condition is low.

- Addition was made to the efficacy analysis, that calculation of estimates other than the time to event analysis is performed for two types of cases as a set: a case when all subjects in each analysis set are targeted and a case when only subjects whose final data has been evaluated in each analysis set are targeted (ie, a set of subjects excluding those whose data after death or event onset has not been measured).

- The multiplicity was to be adjusted in all efficacy analyses. However, the multiplicity is now adjusted only in the primary analysis test.

- It was stated that the efficacy analysis for ALSFRS-R, etc. was analysis using the Wilcoxon rank sum score. However, "rank sum" was deleted to clarify that the analysis uses the Wilcoxon score.

- The PPS criterion, "Subjects who discontinued the study and participated in the study for less than 52 weeks," was deleted. However, due to conducting landmark analysis beginning at Week 52 after registration, the influence on subjects who discontinued/completed early within 52 weeks is to be evaluated, and thus analysis was added.

- Summary of events by type, summary of the frequency of completion of treatment for the study period, and summary of the ratio of subjects adopted to each analysis set were added to the summary of subject disposition.

- %FVC at the start of the observation period was added to the summary items for the baseline characteristics.

- The final date of prescribed injection in the calculation of the applicable period (weeks) by the treatment compliance formula was defined, and the details were added.

- In the primary analysis, the censoring date for subjects who completed the treatment was set to be Week 180 (Day 1261), which is the lower limit of acceptable period for evaluation at Week 182 after treatment, to maintain consistency among subjects. Also, estimated period until event onset (25%, 50%, and 75% event occurrence periods, and hazard ratio) were added. Additionally, narratives on Kaplan-Meier curve combining the placebo group and the active drug group and a hazard ratio for the average contrast (-2, 1, 1) were added.

- The summary of frequency of each question item of ALSFRS-R was changed to calculation of summary statistics of scores by 3 items (bulbar function, limb function, and respiratory function) and between-group comparison (test of contrast using the Wilcoxon score). Calculation of summary statistics for slope of ALSFRS-R was added.

- Calculation of summary statistics for SpO_2 during sleep by treatment group for each evaluation time point was added. Additionally, exploratory analysis is to be conducted to explore the influence on events using proportional hazard model including several thresholds for ratios of 88% or below as time dependent covariates.

- Categorical analysis for the duration of exposure (more than 52 weeks, more than 104 weeks, and more than 156 weeks) was added.

- Regarding the categories for subgroups of adverse event, the initial symptoms, arm onset and leg onset, were combined as limb onset.

8 DEFINITIONS AND CONVENTIONS FOR DATA HANDLING

Data are handled as follows.

<Handling of missing value>

In order to specify handling methods by item, the items for complementing missing values are described in the “Handling of data at each evaluation time point” below.

If one of the question items of ALSFERS-R and ALSAQ-40 is missing, the total score is handled as a missing value. However, it may be included in the summary if calculation of the subtotal is allowed.

<Handling of measured values outside the specified acceptable periods>

The acceptable periods for the efficacy endpoints (ALSFRS-R, %FVC, MMT, grip test, Norris scale, ALSAQ-40) and the safety endpoints (laboratory values, vital signs) are ± 1 week of Week 4 of the treatment period and ± 2 weeks of Weeks 16, 28, 40, 52, 64, 76, 88, 100, 112, 124, 136, 148, 160, 172, and 182 after the treatment thereafter.

Data outside these acceptable periods are handled as missing values. If multiple data exist within these periods, the absolute values of the difference in the number of days with the number of days of the prescribed evaluation are calculated, and the data with the smallest absolute value is adopted as data for the particular evaluation time point. If the absolute values are the same, data is examined individually (by endpoint). This handling does not apply to data from follow-up investigation.

The acceptable periods for SpO_2 during sleep is from the start of the observation period until the end of the observation period, and ± 1 week of Weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, and 182 after the treatment thereafter.

Data outside these acceptable periods are handled as missing values. Additionally, SpO_2 data evaluated after an event (full-time wearing of non-invasive ventilation or wearing of invasive ventilation) is excluded because the intervention by the event may significantly influence SpO_2 data. If multiple data exist within these periods, data evaluated last in the acceptable period of the prescribed evaluation is adopted as data for the particular evaluation time point.

<Handling of measured values for reasons other than deviation from acceptable period>

Data obtained at evaluation time point is excluded from PPS analysis if it is considered to be influenced by violation of dose and schedule or concomitant drugs/therapies even if the data is within acceptable range.

<Handling of outliers>

Based on the results of dry run before database lock, it is determined that there is no specific outlier, and thus variables are not to be changed to process outliers.

<Handling of data at each evaluation time point>

In order to evaluate the influence on subjects who experienced an event, data of the efficacy endpoints (ALSFRS-R, %FVC, MMT, grip test, Norris scale, ALSAQ-40) after event occurrence that was measured last after the study drug treatment is supplemented to all subsequent evaluation time points at which values are missing using Last Observation Carried Forward (LOCF).

Whether to adopt efficacy data at each evaluation time point in PPS was determined by handling of subjects' data based on review by the case conference.

Missing data for the safety endpoints (vital signs, laboratory test values) is not supplemented.

<Handling at final evaluation>

As with "<Handling of data at each evaluation time point>," data of the efficacy endpoints (ALSFRS-R, %FVC, MMT, grip test, Norris scale, ALSAQ-40) after event occurrence that is supplemented by data measured last after the study drug treatment using Last Observation Carried Forward (LOCF) to set the value at the final time point. More specifically, for subjects who completed the treatment period, Week 182 of treatment is regarded as the final time point, and data measured last after the study drug treatment is handled as data at the final time point if an event was observed and the efficacy endpoints were measured after event occurrence.

The Wilcoxon score is calculated for the amount of change. For subjects who were not evaluated after event occurrence (no final time point) and subjects who died, the score is calculated by supplementing with the worst value of the amount of change for each subject. The worst value is -999.

Values at the final time point for the safety endpoints (vital signs, laboratory test values) are also set using LOCF by supplementing with data measured last after the study drug treatment. "Last" means the last data within the allowance of the final evaluation day.

<Handling of data of subjects who discontinued the study>

Data of subjects who discontinued the study are handled as censored data. However, if an event occurred within 28 days after the last day of study drug treatment (the last day of study drug treatment as Day 0) in a subject who discontinued the study, the event and the efficacy data measured after event occurrence and within 28 days after the last day of study drug treatment is used for analysis.

For subjects who discontinued the study, the evaluation at discontinuation is regarded as data at the final time point, and if the data at discontinuation was not evaluated, data measured last after the study drug treatment is regarded as data at the final time point.

<Sample abnormality items in laboratory test>

The case conference extracted items in the laboratory test comment mentioning "hemolysis," "milky fluid," or "agglutination." They were included in summary since measured data were also obtained together. On the other hand, the case conference also extracted items mentioning "coagulation." However, measured data were not obtained, and therefore, the case conference handled data with "coagulation" were handled as missing.

<Handling of grip strength data>

Data >0 kg but <5 kg is handled as 0 kg.

9 PROGRAMMING SPECIFICATIONS

The definition document for data set for analysis is specified separately from this analysis plan.

10 STATISTICAL SOFTWARE

Analysis is conducted by Bell Medical Solutions, Inc. by double programming using SAS for Windows (release 9.2 or later), NONMEM, and Microsoft Excel. In cases of adding analysis not specified in the analysis plan, measures to ensure reliability is considered separately.

11 MOCK TABLES, LISTINGS AND GRAPHS (TLGS)

Forms for the results of analysis for the study are specified separately from the analysis plan.

12 REFERENCES

Not applicable.