Clinical Study Protocol

Japanese Early-stage Trial of high-dose methylcobalamin for Amyotrophic Lateral Sclerosis (JETALS)

Study No.: E0302-TOK-763
Study Phase: Phase III
Investigational product: E0302
Indication: Sporadic ALS or familial ALS

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91
92

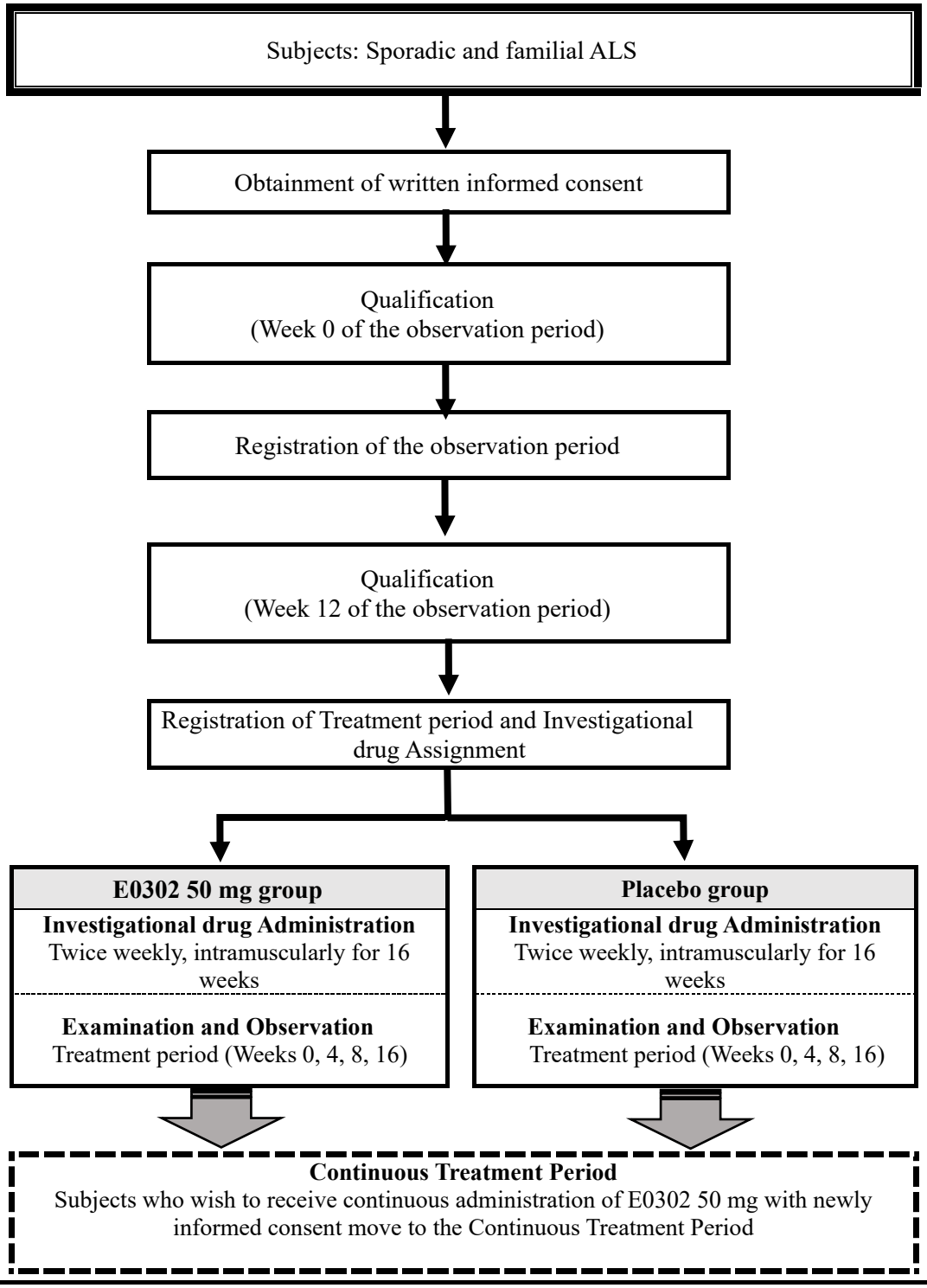
List of abbreviations

Abbreviations	Non-abbreviated
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
BUN	Blood Urea Nitrogen
CK	Creatine Kinase (creatine phosphokinase)
CMAP	Compound Muscle Action Potential
COPD	Chronic Obstructive Pulmonary Disease
FAS	Full Analysis Set
FVC	Forced Vital Capacity
GAD	Gracile Axonal Dystrophy
GCP	Good Clinical Practice
γ-GTP	γ-Glutamyl Transpeptidase
IVH	Intravenous Hyperalimentation
LDH	Lactate Dehydrogenase
MMRM	Mixed-effects Model with Repeated Measurements
MMT	Manual Muscle Testing
MRC score	Medical Research Council score
PEG	Percutaneous Endoscopic Gastrostomy
PPS	Per Protocol Set
PT	Preferred Term
SOC	System Organ Class
SOD1	Superoxide Dismutase 1
WFN	World Federation of Neurology

TABLE OF CONTENTS

Overview

[Schema]



[The purpose of this clinical trial]

To evaluate the superiority of high-dose intramuscular E0302 (mecobalamin 50 mg) compared with placebo in amyotrophic lateral sclerosis (ALS) patients, as an indicator of the Japanese version of the Revised ALS Functional Rating Scale (ALSFRS-R).

[Subjects]

Eligible patients: Those who meet the following inclusion criteria (1)-(4), (6) and (7) at the initiation of the observation period, and (3) and (5)-(7) at the completion of the observation period, and do not meet any of the exclusion criteria (1)-(14) at the initiation of the observation period, and (1)-(4), (7)-(11), (13)-(14) at the completion of the observation period.

Inclusion criteria: Patients who meet all of the following criteria will be included.

(1) Patients who provide written informed consent to participate in the study.
(2) Patients aged 20 years or older at the time of obtainment of informed consent
(3) Patients diagnosed with isolated or familial ALS who meet the definite, the probable, or the probable-laboratory-supported updated Awaji criteria
(4) Patients within one year of disease onset at the initiation of the observation period
(5) Patients with 1-to 2-point decrease in the ALSFRS-R total score during the observation period (12 weeks)
(6) Patients with severity 1 or 2 according to the severity criteria of ALS
(7) Ambulatory patients

Exclusion criteria: Patients who meet any one of the following criteria will be excluded.

(1) Patients undergoing tracheostomy
(2) Patients with a history of non-invasive respiratory support
(3) Patients with % Forced vital capacity (%FVC) ≤ 60%
(4) Patients with chronic obstructive pulmonary disease (COPD)
(5) Patients with neurological symptoms due to vitamin B12 deficiency
(6) Patients receiving edaravone within four weeks prior to enrollment in the observation period
(7) Patients receiving riluzole or have changed or discontinued the dosage after obtaining informed consent
(8) Patients with cognitive impairment
(9) Pregnant women or patients who may be pregnant
(10) Patients with serious respiratory, cardiovascular, or hepatorenal disease
(11) Patients with malignancies
(12) Patients who participated in another clinical trial within 12 weeks prior to the acquisition of consent
(13) Patients with a history or concomitant history of drug allergy or severe allergic disease (anaphylactic shock, etc.)
(14) Patients for whom the principal investigator or sub-investigator judges their participation in the study to be inappropriate

[Sample Size and Duration]

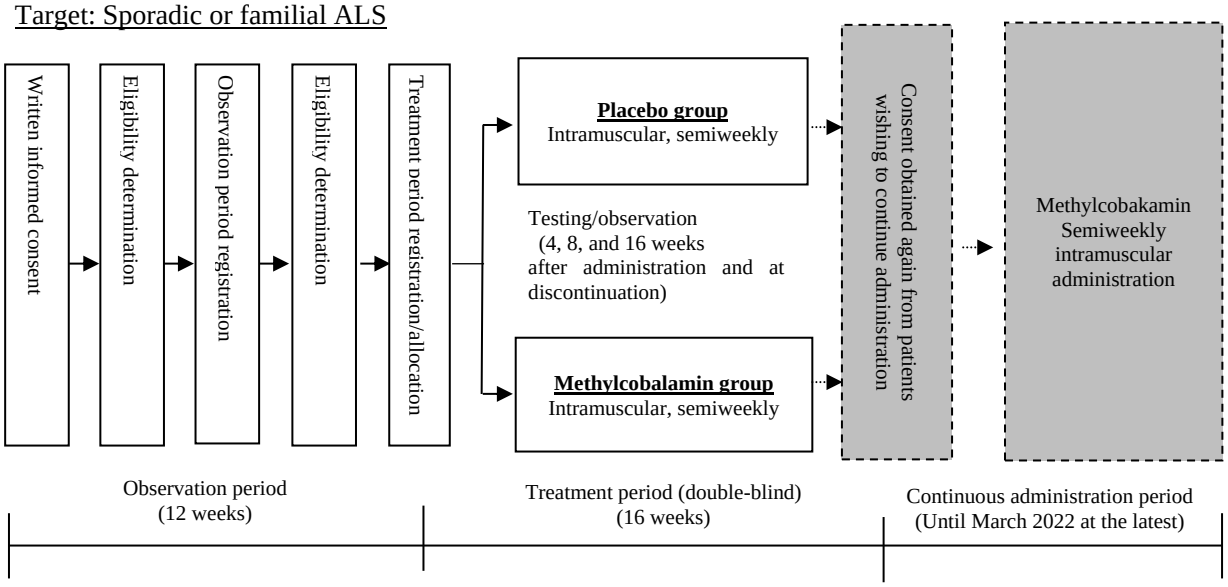
The target number of subjects: 128 (64 in the placebo group and 64 in the E0302 - 50 mg group)
Planned study period: October 2017 to March 2022 (planned)
(If no significant difference is found in the primary endpoint, the study will be terminated at that time.)
Planned period for case registration: October 2017 to October 2019 (planned)
Period for each subject: From the date of obtainment of informed consent to Week 16 of the Treatment period (subjects wishing to continue treatment beyond Week 16 move to the Continuous treatment period (until March 2022) after obtaining informed consent.

[Design of clinical trials]

A multicenter, randomized, placebo-controlled, double-blinded, parallel-group-controlled trial.

Study schedule

Target: Sporadic or familial ALS



[Investigational drug]

Investigational product: E0302 (generic name: mecobalamin)
Control: Placebo (white lyophilized mass or powder without mecobalamin)

[Dosage and method of administration]

E0302 - 50 mg or placebo will be administered intramuscularly twice weekly from the initial day of administration until the completion of the 16-week Treatment period.
Subjects who wish to continue treatment beyond Week 16 of the Treatment period will enter the Continuous treatment period and receive an intramuscular dose of E0302 - 50 mg until March 2022.

[Endpoint]

1) Efficacy endpoint
Main outcome measurements:
Change in the ALSFRS-R total score from the date of randomization to Week 16 of treatment
Secondary outcome measurements:
Change in %FVC, change in the concentration of homocysteine in the blood, change in Manual muscle tet (MMT) total score, change in grip strength (right and left, respectively), change in Norris scale total score, and change in ALS Assessment Questionnaire-40 (ALSAQ 40) total score from the date of randomization to event (all-day wear of non-invasive respiratory support device, wear of invasive respiratory support device, or death).
2) Safety endpoint
Adverse events, laboratory tests, and vital signs.
3) Endpoints in the Continuous treatment period
Time from the date of allocation to the event (all-day non-invasive respiratory support, invasive ventilator placement or death), change in ALSFRS-R total score, laboratory tests, vital signs, adverse events.

[Statistical Methods]

1) Efficacy analysis
The superiority of the treatment in terms of the primary endpoint (i.e., change in ALSFRS-R total score from the date of randomization to 16 weeks of treatment) to placebo will be tested. In the Mixed-effects Model with

Repeated Measurements (MMRM) analysis, a linear model is fitted with the covariance structure of error variance as the unstructured (unstructured) with the fixed effect in the treatment group, time point, minimization factor, and interaction of the treatment group and time point as the covariate of ALSFRS-R total score of the date of allocation as the covariate. Differences are considered significant if the lower boundary of the 95% least-squares mean confidence interval for the change in ALSFRS-R total score at Week 16 compared between the placebo and E0302-50 mg groups is >0. Changes in ALSFRS-R at Weeks 4 and 8 of the Treatment period will be tested according to Week 16. Time-course graphs of least squares mean ± SEM obtained by MMRM analysis are also plotted for each group. Summary statistics for change from the date of allocation for each group and time point (including worst time) will also be calculated.

For the time to event of the secondary endpoint, the log-rank test p-value will be calculated for comparison between groups. Survival curves will be calculated for each group using the Kaplan–Meier method. Survival rates at each time point will be calculated with standard error using Greenwood's formula (as appropriate) to calculate the corresponding 95% confidence interval. For each time point, tests and analyses will be performed according to the primary endpoints for changes in the concentration of homocysteine in the blood, change in %FVC, change in MMT total score, change in grip strength (right and left, respectively), change in Norris scale total score, and change in ALSAQ 40 total score.

2) Safety analysis

The number of subjects with adverse events/reactions and the number of adverse events will be tabulated for each treatment group, and the incidence of adverse events/reactions will be calculated. In addition, the placebo group and the E0302-50 mg group will be compared for the subject-specific incidence of adverse events/adverse reactions using Fisher's exact test, and analyses will be performed for causality and severity.

For laboratory values, the incidence of abnormal changes will be calculated for each treatment group, and the placebo group will be compared with the E0302-50 mg group using Fisher's exact test. For laboratory values and vital signs, the change (or change) from the values at Week 12 for each treatment group will be calculated and compared within groups using Wilcoxon signed-rank test (for one sample). The placebo group will be compared with the E0302-50 mg group using the Wilcoxon rank-sum test (two samples).

1. OBJECTIVE

To evaluate the dose-response to treatment with E0302 and verify its superiority over placebo by conducting a multicenter, randomized, parallel-group, double-blinded, comparative study in patients with ALS, regarding a variation in the Japanese-language version of the ALS Functional Rating Scale-Revised (ALSFRS-R). The safety of E0302 will also be examined.

2. HISTORY OF DEVELOPMENT (BACKGROUND INFORMATION)

ALS is an intractable neurodegenerative disease of unknown origin that progressively presents various symptoms through the failure of upper and lower motor neurons and skeletal muscle force lowering. It develops until the spontaneous motor function, including respiratory function, is completely lost. Usually, loss of respiratory function or severe dysphagia results in death within 3–6 years from the time of onset. Presently, there is no established treatment for this disease.

The pathomechanisms of ALS are only partially understood. The only known cause of ALS is mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene.[1] Mutation in the SOD1 gene result in the expression of mutant proteins in motor neurons. However, approximately 1% to 2% of all patients with SOD1 mutations have ALS, accounting for approximately 20% of familial ALS cases. The molecular mechanism of the disease state of familial ALS caused by mutations in the SOD1 remains unknown.

In addition, the prevalence of ALS is 2-7 per 100,000 population, and the incidence is approximately 1 per 100,000 population.[2] The number of patients identified in Japan was 9,434 (the number of certificates issued for patients receiving treatment for specified diseases in FY 1995).[3]

Currently, riluzole and edaravone are the only approved drugs worldwide for the treatment of ALS. A meta-analysis of clinical trials investigating riluzole conducted in the United States and Europe showed a survival benefit of approximately three months in the riluzole arm. On the other hand, a clinical trial conducted in Japan did not show a significant difference in the time to a certain disease progression (independent ambulation, abolition of arm function, tracheostomy, respirator placement, tube feeding, or death) between the riluzole and placebo groups and failed to demonstrate efficacy. However, in view of the results of clinical trials in Europe and the United States and the social necessity, the drug received early conditional approval in December 1998.[4] Edaravone exhibits free radical-scavenging action, and the improvement on the neurological syndrome, activity of daily living failure, dysfunction with the cerebral infarction acute phase was already manufactured as the indication, and the effect which protects the motoneuron by suppressing the generation of the rising free radical in the disease state of ALS, and delays the progress of the muscle atrophy was expected, and the clinical trial was conducted. In clinical trials, significant differences were observed between the placebo and drug groups in the degree of change in ALSFRS-R scores, an indicator of functional impairment. The results of time-course evaluations showed that the progression of functional impairment could be expected to be delayed by approximately two months. Therefore, it was approved in June 2015 under the "Conditional Approval." [5]

Numerous drugs have been evaluated in clinical trials in patients with ALS. However, with the exception of riluzole and edaravone, none of those have been approved. There is hope for the development of agents that prolong patient survival or improve clinical symptoms.

Mecobalamin is an active form of vitamin B12 that has been used in Japan at an adaptive dose of 0.5 mg once as a treatment for peripheral neuropathy and megaloblastic anemia. On the other hand, *in-vitro* and *in-vivo* studies have suggested that high doses of mecobalamin may exert a protective effect against neurodegeneration. Long-term treatment of cultured cerebral cortical nerve cells and cultured retinal nerve cells with mecobalamin inhibited glutamate-induced neuronal cell death.[6, 7] In addition, in the acrylamide neuropathy model, faster recovery of compound muscle action potentials (CMAP) was observed in the high-dose mecobalamin group compared with the control (saline) group. However, the recovery rate reported in the low-dose mecobalamin group did not differ from that observed in the control group.[8] Furthermore, it was suggested that mecobalamin promoted regeneration of degenerating motor nerve terminals in GAD mice.[9]

Some clinical studies have suggested a clinical effect of high-dose mecobalamin in patients with ALS.[10-12] Following repeated intramuscular administration of mecobalamin at 0.5 or 25 mg/day for 14 days in 24 ALS patients, CMAP amplitudes at four weeks (2 weeks after completion of treatment) were significantly

increased in the 25 mg group compared with baseline (P = 0.038, paired t-test)10. When mecobalamin was administered intramuscularly at doses of 0.5 or 50 mg/day for 14 days in 21 patients with ALS, there was a tendency toward efficacy in the 50 mg group (P = 0.056, paired t-test) in the modified Medical Research Council score (MRC score) after six weeks (4 weeks after completion of administration).[11] In addition, 41 patients with ALS received mecobalamin 50 mg (repeated IM twice weekly) versus no mecobalamin in an open, non-randomized, controlled trial. Mean survival or time to ventilator use was 14.7 ± 11.7 months in the 50 mg group (18 subjects) versus 17.7 ± 9.0 months in the untreated group (16 subjects). However, the Kaplan–Meier survival curves tended to be better in the 50 mg group (February 2002, P = 0.0858, log-rank test).[11] Although all patients in the non-treatment group expired, 11 patients in the 50 mg group continued to receive treatment. The mean survival time or time to respirator use was significantly longer in the 50 mg group at the end of April 2004 (27.7 ± 19.8 months) (P = 0.023, log-rank test).[12]

On the basis of the above-mentioned non-clinical and clinical research results, high-dose mecobalamin is a potentially useful therapy in the treatment of ALS.

In preclinical safety studies (single-and repeated-dose toxicity), mecobalamin was not severely toxic in any species, and there were no relevant findings in terms of genotoxicity, reproduction/developmental toxicity, or antigenicity. In safety pharmacological studies, there were no adverse effects observed on the central nervous system, cardiovascular system, or respiratory system.

A Phase I single-dose study performed in the United Kingdom (E0302-E044-001) demonstrated the safety and tolerability of single intramuscular administration of mecobalamin at doses of 25, 50, or 75 mg in healthy Japanese and Caucasian male and female adults. Single-dose pharmacokinetic parameters showed dose linearity, and pharmacokinetic parameters were similar between the Japanese and Caucasian participants. Subsequently, a Phase I repeat-dose study also performed in the United Kingdom (E0302-E044-002) demonstrated the safety and tolerability of repeated intramuscular administration of mecobalamin at doses of 25 or 75 mg in healthy Japanese and Caucasian male and female adults for seven days. Pharmacokinetic parameters after repeated administration showed similar results to those obtained from the single-dose study. There was no accumulation observed after repeated administration.

On the basis of the above evidence, high-dose mecobalamin may be useful in the treatment of ALS, and the safety and pharmacokinetic results were favorable in Phase I clinical studies. A Phase II/III study (E0302-J081-761) involving Japanese ALS patients was conducted by Eisai Co., Ltd. (hereinafter Eisai Co., Ltd.). The results of this study failed to show superiority in terms of the primary efficacy endpoint (change in ALSFRS-R total score from the completion of the observation period to Week 16 of the Treatment period), although there was a trend toward greater efficacy in the mecobalamin 25 mg and 50 mg groups versus the placebo group. On the other hand, in the subgroup of subjects with ALS onset ≤12 months (48 subjects in the placebo group, 54 subjects in the 25 mg group, and 42 subjects in the 50 mg group), dose-response prolonged the time to the event and reduced the ALSFRS-R total score. However, the results of the subgroup analyses, the high efficacy in ALS patients who were diagnosed early and enrolled in the study were considered to be clinically meaningful. In addition, one death from cardiac arrest was reported as a serious adverse reaction in the mecobalamin 50 mg group. The event was considered by the investigator to be "due to myocardial infarction, arrhythmia, etc., but it was difficult to determine the cause of death. The event was most likely not related to the study treatment but occurred during the study treatment period. The event could not be completely ruled out." The event was considered "possibly related to the investigational drug." On the contrary, the sponsor considers it difficult to assess the causal relationship to the investigational drug because the cause of death has not been identified. In that study, although the incidence of adverse events was high, the incidence of adverse reactions was limited. Moreover, there were no obvious differences in the incidence of adverse events or adverse reactions between the placebo, 25 mg, and 50 mg groups, and there were no problems associated with the safety of intramuscular administration of mecobalamin 25 mg or 50 mg. In addition, a Phase III clinical study (E0302-J081-762) was conducted by Eisai Co., Ltd. as an extension study of Phase II/III study (E0302-J081-761) to investigate the safety and efficacy of continuous long-term administration of E0302 - 50 mg in ALS patients. From the results of the 52 weeks assessment by the cut-off point (31 October 2014), there was no particular concern regarding the safety of long-term administration. The results of survival, cumulative event rate, ALSFRS-R, and %FVC suggested that E0302 maintains its inhibitory effect on the progression of ALS.

On the basis of the results of Phase II/III study conducted by Eisai Co., Ltd., we planned to perform a multicenter, randomized, placebo-controlled, double-blinded, parallel-group study with the primary endpoint of change in the ALSFRS-R total score from the date of allocation to Week 16. The study will include patients with ALS who had developed ALS within one year after the onset of symptoms at the initiation of the observation period and had a 1-2-point decrease in the total ALSFRS-R score during the observation period (12 weeks). In addition, subjects who wish to continue treatment beyond Week 16 of the Treatment period were allowed to continue treatment with E0302 - 50 mg by moving to the Continuous treatment period until March 2022.

3. STUDY DESIGN

3.1. Study Design

A multicenter, randomized, placebo-controlled, double-blinded, parallel-group-controlled trial
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3.2. Study Flow

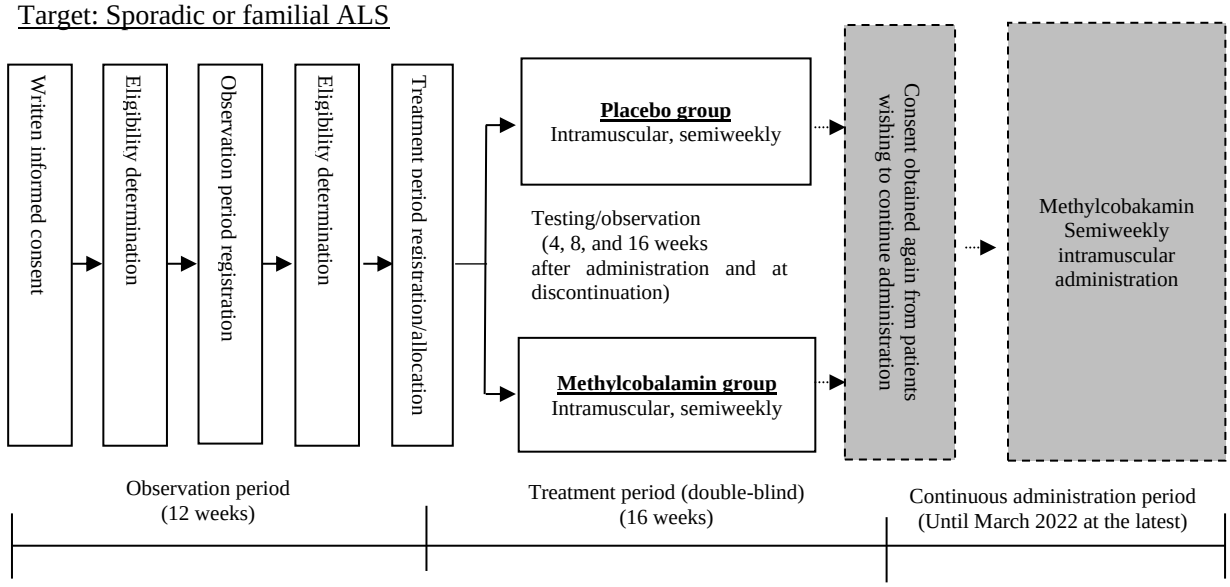

Figure 3-1 Schematic of Study Conduct

This study consists of three periods: the observation period, Treatment period, and Continuous treatment period (see Figure 3-1 Summary of Study Conduct).

The investigator or sub-investigator will determine eligibility at the initiation of the observation period and enroll subjects in the 12-week observation period following the obtainment of written consent to participate in the study (see section 8.2.1 "Procedures for Subject Enrollment and Assignment of Investigational Products"). The investigator or sub-investigator will determine eligibility at the completion of the observation period after a 12-week follow-up period.8.2.1

The registration center will assign subjects who are eligible at the completion of the observation period to either the placebo or E0302 - 50 mg groups by dynamic allocation (see 8.2.2 "Assignment Procedure"). These patients will be enrolled in the Treatment period.

The Treatment period is 16 weeks. During this period, to ensure blinding, the administration of the investigational drug, efficacy evaluation, safety evaluation, and ALSFRS-R evaluation will be performed by independent personnel at the study site. The evaluators of the efficacy, ALSFRS, and safety will perform the required assessments at 4, 8, and 16 weeks after the initiation of treatment, at the time of the

event, or at the time of discontinuation. The Continuous treatment period shall be until March 2022. In the Continuous treatment period, independent evaluation is not required, and all evaluations, surveys, and administration of the investigational product are permitted by the principal investigator and others.

3.3. Discussion of Study Design, Including the Choice of Control Groups

1) Reason for the placebo-controlled comparative study

Riluzole and edaravone have been approved for the treatment of ALS. A meta-analysis of clinical trials investigating riluzole conducted in Europe and the United States showed a survival benefit of approximately three months in the riluzole group. However, a clinical trial conducted in Japan failed to demonstrate efficacy, without significant difference observed in the time to disease progression (independent ambulation, abolition of arm function, tracheostomy, respirator placement, tube feeding, or death) between the riluzole and placebo groups. However, in view of the results of clinical trials in Europe and the United States and the social necessity, the drug received early conditional approval in December 1998. In light of this background, the efficacy of riluzole has not been clarified, at least in Japan. Although edaravone was approved in June 2015, the number of ALS cases treated with this drug has not been determined, and thus, it is difficult to state that edaravone has been established as a standard treatment for ALS. Therefore, it is considered inappropriate as a comparator in this study. Hence, it is reasonable to conduct this investigation as a placebo-controlled study to verify the efficacy of E0302 objectively. In addition, since significant difference versus placebo could not be shown in the clinical Phase II/III study of E0302, it was judged necessary to demonstrate the superiority to placebo again. Thus, the placebo was set as a control. However, considering the ethical aspects of treatment, the subjects receiving riluzole at the time of obtainment of informed consent will be allowed to continue treatment, provided that its daily dose is not changed from the time of informed consent obtainment to the completion of the Treatment period or at the time of discontinuation, in principle.

2) Reasons for setting the observation period prior to commencement of administration of the investigational drug

ALS is a progressive disease. This clinical study evaluates the inhibitory effect of E0302 on the progression of ALS. Because of individual differences in the extent of ALS progression, homogenization of subject characteristics is required to evaluate the efficacy of the drug adequately. For this reason, a 12-week observation period was set prior to the initiation of drug administration to investigate the subject's clinical symptoms and the extent of progression.

3) Measures to ensure blinding

The investigational drug product E0302 is characterized by a red color attributable to the drug substance. In addition, it is expected that the urine of subjects treated with E0302 will be red. Therefore, as a measure to ensure the blinding of the study and reduce assessor bias, efficacy assessors, ALSFRS-R assessors, safety assessors, and investigational drug administrators at participating medical organizations are assigned to different personnel (see 8.3.4 "Blinding of Investigational drug and Urine Color").

The system required for conducting the study at the participating medical organizations

(1) Efficacy evaluator (at least one)
(2) ALSFRS-R assessors (at least one)
(3) Safety assessor (at least one)
(4) Subjects receiving the investigational product (at least one)

3.4. Sample Size and Study Period

The target number of subjects: 128 (64 in the placebo group and 64 in the E0302 - 50 mg group)
Study period: October 2017 to March 2022 (planned)
(If no significant difference is found in the primary endpoint, the study will be terminated at that time.)
Case registration period: October 2017 to October 2019 (planned)
Period for each subject: From the date of informed consent obtainment to Week 16 of the Treatment

period (subjects wishing to continue treatment beyond Week 16 move to the Continuous treatment period (until March 2022) after obtaining informed consent.

[Rationale]
The rationale for sample size selection is described in section 18.1, "Determination of sample size."

4. SELECTION OF STUDY POPULATION

4.1. Diagnostic criteria

Patients with isolated or familial ALS who meet the definite, probable, or probable laboratory-supported criteria listed in the updated Awaji Criteria[13] (Table 4-1) are eligible for this study. Signs of upper and lower motor neuron damage[14] are shown in Table 4-2. Diagnostic criteria for needle electromyography used in the updated Awaji criteria[15] are shown in Table 4-3.

TABLE 4-1 Updated Awaji Criteria

Diagnostic grade
Definite
○Clinical or neurophysiological evidence of upper and lower motor neuron dysfunction in the bulbar region and at least two spinal regions, or three spinal regions
Probable
○Clinical or neurophysiological evidence of upper and lower motor neuron dysfunction in at least two regions with some upper motor neuron signs necessarily rostral (above) to lower motor neuron dysfunction
Probable laboratory-supported
○Clinical signs of upper and lower motor neuron dysfunction in one region together with neurophysiological evidence of lower motor neuron dysfunction in 2 regions
Possible
○Clinical or neurophysiological evidence of upper and lower motor neuron dysfunction in one region, or Upper motor neuron signs evident in 2 regions, or lower motor neuron dysfunction evident rostral (above) to upper motor neuron signs

Table 4-2 Signs of upper and lower motor neuron damage

	Brainstem	Cervical	Thoracic	Lumbosacral
Lower motor neuron sign				
weakness, atrophy, fasciculations	jaw, face, palate, tongue, larynx	neck, arm, hand, diaphragm	back abdomen	back, abdomen, leg, foot
Superior neuronal sign				
pathologic spread of reflexes, clonus, etc.	clonic jaw jerk, gag reflex, exaggerated snout reflex, pseudobulbar features, forced yawning, pathologic deep tendon reflexes (DTRs), spastic tone	clonic DTRs, Hoffmann reflex, pathologic DTRs, spastic tone, preserved reflex in weak wasted limb	loss of superficial abdominal reflexes, pathologic DTRs, spastic tone	clonic DTRs, estensor plantar response, pathologic DTRs, spastic tone, preserved reflex in weak, wasted limb

BR Brooks et al. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000 Dec;1(5):293-299.

TABLE 4-Diagnostic Criteria for Needle Electromyography Used for Three Updated Awaji Criteria

(If A and B are satisfied, each muscle fulfills the criteria, and if the required number of muscles is satisfied, the criteria for each nerve site are fulfilled.)

Chronic neurogenic changes (A)	Active neurogenic changes (B)
Increased amplitudes, prolonged duration, and the appearance of multiphasic waves of motor unit potentials (motor unit potential)	Positive sharp wave (sharp positive wave)
Delayed MUP recruitment	Fibrillation potential (Fiber Power Generation)
The unstable and complex waveform of MUP	Fasciculation potential (fascicular self-generation) (Note: Only in the same muscle with chronic neurogenic changes)

	Number of muscles needed to meet electrophysiological criteria
Cranial nerves	One muscle
Cervical spinal cord	Two muscles
Thoracic spinal cord	One muscle
Lumbosacral spinal cord	Two muscles

De Carvalho M, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol. Excerpt from 2008;119:497-503.

The El Escorial revised Airlie House diagnostic criteria are shown below.[16, 17]

(1) Clinically reliable ALS (clinically definite ALS) is defined as clinical or neurophysiological evidence of upper and lower motor neuron dysfunction in the bulbar region and at least two spinal regions or three spinal regions.

(2) Clinically probable ALS (clinically probable ALS) is defined as clinical or neurophysiological evidence of upper and lower motor neuron dysfunction in at least two regions with some upper motor neuron signs

492 necessarily rostral (above) to lower motor neuron dysfunction.
493 (3) Clinically probable and laboratory evidence of ALS (clinically probable-laboratory-supported ALS) is
494 defined as clinical signs of upper and lower motor neuron dysfunction in one region together with
495 neurophysiological evidence of lower motor neuron dysfunction (Positive sharp wave, Fibrillation
496 potential) in 2 regions.
497 (4) Clinically possible ALS (clinically possible ALS) is defined as clinical or neurophysiological evidence
498 of upper and lower motor neuron dysfunction in one region, or Upper motor neuron signs evident in 2
499 regions, or lower motor neuron dysfunction evident rostral (above) to upper motor neuron signs.
500 (5) Clinically suspected ALS (clinically suspected ALS) presents with pure lower motor neuron involvement
501 and is not suitable as a group for the purpose of the clinical study of ALS. Therefore, it is excluded from
502 the global neurology association El Escorial revised ALS diagnostic criteria.
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504

4.2. Severity Criteria for ALS

The severity of ALS is assessed on the basis of ALS severity criteria 2) shown in Table 4-4. In this clinical trial, patients who require assistance, orthoses, or assistive devices but whose daily life and work are judged to be self-sufficient will be included in severity 2.

Table 4-Criteria for Severity of 4 ALS (Ministry of Health and Welfare, Research Group on Neurodegenerative Diseases, 1998)

Severity	Criteria
First degree	Dysarthria due to movement of one limb or bulbar palsy but does not interfere with daily life or employment
Second degree*	Among the six muscles of each body and extremity (4), muscle of the trunk (1), tongue, face, palate, and pharynx (1), there are obvious motor disorders in one or two parts of the body and extremities, and thus there is a living disability. However, it is possible to live and work independently.
Third-degree	Since the muscle force lowering of three or more parts in the above six positions cannot continue the social life such as housework and employment, and assistance is needed for daily life.
Fourth degree	Inability to breathe, swallow, or maintain a sitting position, necessitating assistance in all aspects of daily life
Fifth degree	Bedridden and require full life support

※: This study will include patients who require assistance, orthoses, or assistive devices but whose daily life and employment are judged to be almost independent and possible.

4.3. Eligibility Criteria

Patients who meet the following inclusion criteria (1)-(4), (6) and (7) at the initiation of the observation period, and (3) and (5)-(7) at the completion of the observation period, and do not meet any of the exclusion criteria (1)-(14) at the initiation of the observation period, and (1)-(4), (7)-(11), (13)-(14) at the completion of the observation period are eligible.

4.3.1. Inclusion Criteria

Patients who meet all the following criteria will be included:

(1) Patients who have provided written informed consent to participate in the study.
(2) Patients aged ≥20 years at the time of obtainment of informed consent.
(3) Patients diagnosed with isolated or familial ALS who meet the definite, the probable, or the probable laboratory-supported of the updated Awaji criteria.
(4) Patients within one year of onset at the initiation of the observation period.
(5) Patients with a 1-2-point decrease in the ALSFRS-R total score during the observation period (12 weeks).
(6) Patients with severity 1 or 2 according to the severity criteria of ALS.
(7) Ambulatory patients.

[Rationale for setting the inclusion criteria]

(1) It was established on the basis of Good Clinical Practice (GCP).
(2) In consideration of obtaining written informed consent from the patient, the patient was considered to be ≥20 years of age.
(3) Updated Awaji criteria with increased diagnostic sensitivity were used in this study to include patients with early-stage disease.
(4) It was set to enroll the patients in the early stages of the disease.
(5) It was set in order to confirm the degree of the progress of the disease state.

(6) (7) It was set for the patients whose daily life and employment were judged to be almost independent.

4.3.2. Exclusion Criteria

Patients who meet any one of the following criteria will be excluded.

(1) Patients undergoing tracheostomy
(2) Patients with a history of non-invasive respiratory support
(3) Patients with %FVC ≤ 60%
(4) Patients with chronic obstructive pulmonary disease (COPD)
(5) Patients with neurologic symptoms due to vitamin B12 deficiency
(6) Patients who have received edaravone within four weeks prior to enrollment in the observation period
(7) Patients receiving riluzole or have changed or discontinued the dosage after obtaining informed consent
(8) Patients with cognitive impairment
(9) Pregnant women or patients who may possibly be pregnant
(10) Patients with serious respiratory, cardiovascular, or hepatorenal disease
(11) Patients with malignancies
(12) Patients who participated in another clinical trial within 12 weeks prior to the acquisition of consent
(13) Patients with a history or concomitant history of drug allergy or severe allergic disease (anaphylactic shock, etc.)
(14) Patients for whom the principal investigator or the sub-investigator judges their participation in the study to be inappropriate.

[Rationale for setting the exclusion criteria]

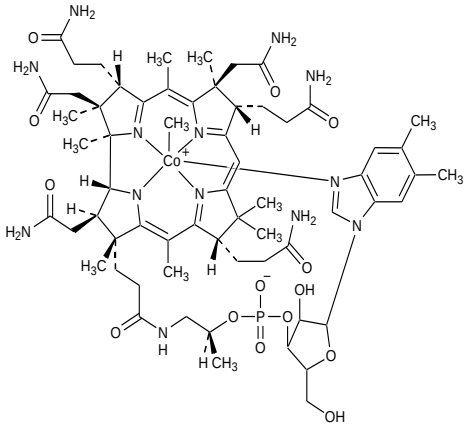
(1)–(4) To exclude patients with decreased respiratory function.
(5) It was set in order to exclude those with sub-acute combined myelodegeneration.
(6) This drug has been designated as a disallowed concomitant drug and included because it is expected to affect the evaluation of the efficacy of the investigational product.
(7) Limited concomitant medication – riluzole – is included because it is expected to affect the efficacy evaluation in this study.
(8) To appropriately evaluate the efficacy and safety.
(9)–(14) It was set considering patient safety.

5. TREATMENT

5.1. Identity of Investigational Product

5.1.1. Chemical Name and Structural Formula of E0302

Investigational drug code: E0302
Generic name: mecobalamin
Chemical Formula: Co-[-(5, 6-Dimethylbenz-1 H-imidazolyl)]-Co-methylcobamide
Molecular formula: C 63 H#2] CoN 13 O 14 P
Molecular weight: 1344.38
Structural formula: mecobalamin



5.1.2. Comparator Drug

Placebo: White lyophilized mass or powder without mecobalamin

5.1.3. Drug Preparation

The E0302 content, color, and dosage form of each drug product are shown in Table 5-1.

Table 5-1 E0320 Content, Color, Dosage Form of Preparations

Investigational drug	Content, color, dosage form
E0302 - 25 mg injection	Red lyophilized mass or powder containing mecobalamin 25 mg in one vial for injection
E0302 Placebo injection	White lyophilized masses or powders without mecobalamin for injection

Storage Conditions: Light-resistant at room temperature.

5.2. Investigational Drug Labeling and Packaging

5.2.1. Packaging

<Investigational product for Treatment period>

E0302- 25 mg injection and E0302 Placebo injection are packaged in the vial of the investigational drug, and the indistinguishability of E0302 - 25 mg injection and E0302 Placebo injection is guaranteed by the appearance. Two vials for one dose are packaged in a small box (one package unit), and 32 small boxes are packaged in a large box (supply for 16 weeks of treatment). A small box containing two vials for a single dose contains one of the following combinations of the investigational product:

- E0302 - 50 mg group: E0302 - 25 mg injection 2 vials

・ Placebo group: E0302 - Placebo injection 2 vials

<Investigational product used in the Continuous treatment period>

E0302 - 25 mg injection shrinks the investigational drug vial. Two vials for one dose are packaged in a small box (one package unit), and 32 small boxes are packaged in a large box (16 weeks' supply). The following combinations of investigational products are packaged in small boxes containing two vials for one dose:

・ E0302 - 50 mg group: E0302 - 25 mg injection 2 vials

5.2.2. Investigational Drug Labeling

Labels for vials, small boxes, and large boxes contain the following information:

<Sample labeling of investigational products for Treatment period>

Study <Treatment period>
Identification No.
Storage
Lot number
Name, title, and address of the sponsor-investigator
Protocol No.
Drug Number (label only for vial not shown)
Expiration date

<Sample label of the investigational product for Continuous treatment period>

For Clinical Trial <Continuation Period>
Identification No.
Storage
Lot number
Name, title, and address of the sponsor-investigator
Protocol No.
Expiration date

5.3. Storage of Investigational Drug

The Supervisor of the Investigational Drug shall appropriately manage the investigational drug in accordance with the "Procedures for Management of the E0302 Investigational Drug" and prepare the Investigational Drug Accountability Record to understand the status of use of the investigational drug and the progress of the study. The investigational product should be stored in a light-resistant container at room temperature.

5.4. Delivery of Investigational Drug to Study Institution

The coordinating investigator (representative, hereinafter the same) will supply the investigational product to the Supervisor of the Investigational Drug. At that time, the Supervisor of the Investigational Drug (or its representative) receives the Investigational Drug Delivery Form and, after confirming the quantity, etc. of the investigational drug, enters the date of receipt, name of the participating medical

organization, and affiliation on the Investigational Drug Receipt Form, and signs and seals or signs.

The Supervisor of the Investigational Drug shall record the status of receipt and export of the investigational drug, the status of use of the investigational drug for each subject, and the recall or disposition of unused investigational drugs in the Investigational Drug Accountability Record. Refer to the "Procedural Manual for the Control of E0302 Investigational Products" for details of records related to the management of the investigational product.

5.5. Administration of Investigational Drug

5.5.1. Dose and Administration Method

E0302 - 50 mg or placebo will be administered intramuscularly twice weekly from the initial day of administration until Week 16 of the Treatment period.

For subjects who wish to enter the Continuous treatment period after the completion of the Treatment period, E0302 - 50 mg will be administered intramuscularly twice weekly from the initial day of the Continuous treatment period until March 2022.

[Justification of dosage]

On the basis of the reported results of clinical studies and the results of Phase II/III study, the doses investigated in this study were E0302 - 50 mg and placebo. The rationale for dose selection is described below.

- In a clinical study, long-term intramuscular administration of 50 mg twice weekly tended to be associated with better Kaplan–Meier survival curves (P = 0.0858, log-rank test).[11]
- In a subgroup analysis in Phase II/III study involving patients within one year from the onset of symptoms, dose-response (monotonic and saturating) was observed in the subgroup of patients for 12 months (P = 0.010 and P = 0.011, respectively; log-rank score versus [-1, 0, 1] and contrast [-2, 1, 1] testing). The hazard ratios (95% confidence intervals) for the placebo group were 0.640 (0.377, 1.085) and 0.498 (0.267, 0.929) for the 25 mg and 50 mg groups, respectively. The time to an event rate of 50% (95% confidence interval) was 570 (465, 720) days, 1087 (564, -) days, and 1197 (609, -) days for the placebo, 25 mg, and 50 mg groups, respectively. In addition, there was also a dose-response (monotonic and saturating) reduction in ALSFRS-R total scores (P = 0.003 and P = 0.013, respectively; contrasts using Wilcoxon scores [-1, 0, 1] and contrasts [-2, 1, 1] tests), and the median (minimum to maximum) change in ALSFRS-R total score was -26.5 (-40 to-3), 25 mg-26.5 (-40 to 0), and 50 mg-22.0 (-38 to 1) in the placebo group. The results of this study suggested that effects are particularly expected in the 50 mg group.
- Due to technical limitations in the design of the formulation, the high-dose formulation that can be manufactured at this time is 25 mg vials, with the highest concentration currently available being at 12.5 mg per mL. For this reason, two intramuscular injections of 2 mL (i.e., total 4 mL) and three intramuscular injections of 2 mL (i.e., total 6 mL) are administered for doses of 50 mg and 75 mg, respectively. In the present study, it is considered difficult to administer >75 mg of the investigational drug because of the patient's suffering.
- The washout period was allowed from the last day of administration to 14 days and 28 days in the Treatment period and Continuous treatment period, respectively, in consideration of the interference with the subjects' daily activities.

5.5.2. Method of Preparation and Administration

The investigational drug administrator will administer the investigational drug twice weekly on an outpatient basis in accordance with the instructions for administration provided by the safety assessor. If the subject becomes unable to visit the hospital as an outpatient, the investigational product may be administered by a coordinator, such as a family physician, who has been designated as an investigational product administrator at the subject's home or nearby medical institution. The investigational product will be administered twice weekly during the 7-day period, starting from the initial day of administration in

the Treatment period and Continuous treatment period. It is not possible to administer two doses (four vials) during the same day with at least one dosing interval.

Two vials will be used per dose for the administration of the investigational product. Immediately prior to the administration of the investigational product, the administrator will add 2.2 mL of isotonic sodium chloride solution to each vial to confirm that the contents have completely dissolved and subsequently administer the 2.0 mL intramuscularly at two locations (i.e., total 4 mL). The drug solution should be used immediately after reconstitution and protected from light. The following precautions should be taken when administering the drug intramuscularly:

- In principle, the application site should be the anterior thigh, lateral thigh, deltoid, or gluteus maximus (or gluteus medius).
- As a general rule, repeated administration at the same site should be avoided.
- The site of application should be avoided.
- If insertion into the injection site causes severe pain, and if blood flows backward, withdraw the needle immediately and inject at a different site.

When administering the investigational product, the investigational drug administrator should provide information on the day of administration of the investigational product, site of administration, drug number, name of the investigational drug administrator, blood pressure prior to administration, pulse rate, and safety in the "In-house E0302 Administration Record Form" or the "External E0302 Administration Record Form."

In addition, two vials should be used at the time of one dose administration. If the administration is not possible due to the subject's condition, details such as the reason for withdrawal will be recorded in the treatment record. The washout period may be 14 days and 28 days, respectively, from the last dosing day in the Treatment period and the Continuous treatment period.

Subjects who have entered the Continuous treatment period will be allowed to receive the investigational product at home (self-administration) by subjects trained in the administration of the investigational product or by their families, in addition to trial collaborators such as their own physicians designated as study medication administrators. When administering the investigational product, the subject or his/her family should provide information on the day of administration of the investigational product, the site of administration, the name of the investigational drug administrator, blood pressure, pulse rate, and safety prior to administration in the E0302 Self-Administration Implementation Sheet.

5.5.3. Treatment Period

The Treatment period is 16 weeks (4 months). In addition, if the subject wishes, the Extension may be continued until March 2022 as the Continuous treatment period.

Even during the Continuous treatment period, treatment will be discontinued at the time of the event.

[Rationale for the setting of dosing period]

In the analysis of the subgroup of Phase II/III study, the difference in the change in the ALSFRS-R total score at four weeks (95% confidence interval) was 0.4 (-0.7, 1.5), which was not significantly different (P = 0.258), but the difference in the change in the ALSFRS-R total score at 16 weeks (95% confidence interval) was 3.3 (0.5, 6.0), which was significant (P = 0.017).

On the other hand, a decrease in the ALSFRS-R total score at 100 days of observation has been reported to be helpful in predicting survival.20 ALSFRS-R assessment at 16 weeks may be predictive of clinically significant survival.

Therefore, it was deemed appropriate to have a 16-week evaluation period, and a shorter 4-week evaluation period would be difficult. Since this study is a placebo-controlled study in subjects with ALS, the Treatment period was set at 16 weeks in order to shorten the placebo administration period as much as possible due to ethical considerations. The extended administration was allowed until March 2022 (Continuous treatment period).

5.6. Storage and Prescription of Investigational Drug

5.6.1. Subjects who can attend the outpatient clinic

In this clinical trial, the investigational product manager will appropriately store and manage the investigational product according to the " Procedures for Management of the E0302 Investigational Drug ". The principal investigator will dispense the investigational product to the investigational product manager. The investigational product manager will dispense the product to the administrator. Subsequently, he/she will administer the drug to the subject.

5.6.2. Subjects who cannot attend the outpatient clinic

5.6.2.1. During the Treatment Period

In principle, during the mid-Treatment period, the drug will be administered to subjects in outpatient clinics. If ambulatory visits become difficult due to reasons such as the progress of the underlying disease, they will be permitted to be administered by the investigational drug administrator at the subject's home or nearby medical institution. In addition, refer to the "Procedures for Delegation of Study Treatment." Storage of the investigational product at the subject's home is permitted. The following precautions should be taken when storing the investigational product at the subject's home.

- When investigational products are stored at the subject's home, the maximum number of small boxes shall be up to 8 per storage.
- The Supervisor of the Investigational Drug shall place the materials necessary for the administration of the investigational drug, needles, syringes, etc., in the special control box and dispense them to the subject or family.
- The investigational products will be locked at room temperature and under light-resistant conditions in a special control box at the subject's home.
- The investigational drug administrator, the subject, or the family should pay attention to the storage conditions of the investigational product.

5.6.2.2. During the Continuous Treatment Period

In this study, when ambulatory visits are difficult due to the patient's reasons, such as the reason for progression of the underlying disease during the Continuous treatment period, because of the patient's health status, etc., administration of the investigational product at the subject's home (self-administration) may be performed by subjects or their families trained in the administration of the investigational product in addition to the administration at the subject's home or nearby medical institutions. In the considerations for storing the investigational products at the subject's house (see "5.6.2.1. During the Treatment Period"), it is not required to lock if it is self-administered. If the investigational products are stored at the subject's home, the maximum number of investigational drugs that can be prescribed is 28.

If the effects of the new coronavirus infection force us to take measures that differ from the provisions of the study protocol and normal procedures, we shall follow the "Procedures for Conducting a Clinical Trial under the Influence of a New Coronavirus Infection," which is provided separately, while placing the highest priority on ensuring the safety of subjects. In providing the investigational drug for administration at the subject's home, the subject may use an investigational drug delivery company to have the drug delivered directly to his/her home. With regard to the delivery of the investigational drug to the subject's home, the investigational drug shall be delivered to the subject without fail in accordance with the "Procedures for Transporting Investigational Drugs to the Subject's Home" separately specified, with due attention to personal information management.

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5.7. Retrieval of Investigational drugs

After completion of the study, the Supervisor of the Investigational Drug shall promptly return the investigational drug (small boxes containing unused and used vials) from the participating medical organization to the coordinating investigator.

5.7.1. Retrieval of Unused and Used Investigational drugs during the Treatment Period

1) Unused
- The person who receives the investigational product shall return the unused investigational product to the Supervisor of the Investigational Drug.

2) Used
- The investigational drug administrator shall return a small box containing used vials to the investigational drug manager.

5.7.2. Retrieval of Unused and Used Investigational drugs during the Continuous Treatment Period

1) Unused
- The person who receives the investigational product shall return the unused investigational product to the Supervisor of the Investigational Drug.
- When self-administering, the subject or family shall place unused investigational products in a special control box and return them to the Supervisor of the Investigational Drug.

2) Used
- The investigational drug administrator shall return a small box containing used vials to the Supervisor of the Investigational Drug.
- In the case of self-administration, the subject or family shall return a small box containing used vials to the Supervisor of the Investigational Drug in the Special Management Box.

6. PRIOR AND CONCOMITANT THERAPY

6.1. Prior Treatment

Except for patients receiving edaravone as prior therapy within four weeks prior to enrollment in the observation period (see "4.3.2. Exclusion Criteria"), no pretreatment provisions will be established.

6.2. Concomitant Therapy

6.2.1. Prohibited Concomitant Drugs and Therapy

The following drugs will be prohibited from the initiation of the observation period to Week 16 of the Treatment period or at the time of discontinuation, whichever comes first.

1) Drugs with Possible Efficacy in ALS (Appendix 2)
2) Drugs whose main ingredient is vitamin B12 (excluding topical agents) (Appendix 2)
3) Other investigational products, investigational products such as regenerative medicine, and investigational devices

[Rationale]

1) (2) Since this product may affect the evaluation of the efficacy of the investigational product, it was set.
3) Considerations for the safety of subjects were included.

The following therapies are prohibited from the initiation of the observation period to Week 16 of the Treatment period or at the time of discontinuation, whichever comes first.

4) HAL medical leg type (Appendix 2)

6.2.2. Restricted Concomitant Drug

Subjects taking riluzole at the initiation of the observation period will be allowed to take further doses. However, the daily dose of riluzole should not be changed or administered at the initiation of the observation period until Week 16 of treatment or at the time of discontinuation, whichever comes first. Dosage reduction or discontinuation is permitted if an adverse event attributable to riluzole occurs or if

the daily dose of riluzole must be changed due to increased dysphagia, etc. Dose escalation or re-administration is not allowed after dose reduction or discontinuation.

[Rationale]

ALS is a fatal disease. In view of the ethical aspects of the subjects, concomitant use of riluzole was allowed. However, because the administration of riluzole is expected to affect the evaluation of the efficacy of the study, a restriction was set regarding the daily dose of riluzole coadministration.

6.2.3. Concomitant Therapy

1) Rehabilitation

Rehabilitation can be performed during all periods of the Observation, Treatment, and Continuous treatment periods. Active rehabilitation should be provided according to the subject's condition, including exercise and respiratory training to restore muscle strength (see 10.7 Concomitant Medications and Concomitant Therapies). However, the HAL medical leg type is prohibited from the initiation of the observation period to week16 of the Treatment period or at the time of discontinuation, whichever comes first.

2) Nutritional management

Even if nutritional support measures (e.g., nasogastric tube feeding, IVH, or PEG) are administered during all periods of the Observation, Treatment, and Continuous treatment periods, a continuation of the study is allowed (see 10.7 Concomitant Medications/Therapies).

7. INFORMED CONSENT

7.1. Preparation of Written Consent Form

The principal investigator will prepare an explanatory document and an informed consent form (form). In addition, the prepared information and informed consent forms (forms) will be submitted to the Institutional Review Board prior to the initiation of the clinical trial for approval.

At a minimum, the explanatory documents should include the necessary information in accordance with the Good Clinical Practice Ordinance (hereinafter referred to as the GCP Ordinance) and related notifications, etc., with reference to the Guidance on Good Clinical Practice.

7.2. Obtaining Written Informed Consent

1） Timing and Methods of Initial Informed Consent

Prior to a patient's participation in the study, the investigator or sub-investigator will fully explain the patient's participation in the study using written information and obtain his or her written consent to participate in the study of his or her own free will. When obtaining written informed consent, the patient fully understands the content of the written information, enters the date on the informed consent form, and signs and seals. The investigator, sub-investigator, or study collaborator who provided the explanation will date and sign or seal the explanation. In addition, the principal investigator, etc., supplies a copy of the signed and sealed consent form to the subject together with the explanatory documents and stores the original consent form with the medical records at the relevant participating medical organization.

If the subject has sufficient capacity to provide informed consent, but it is difficult to sign and seal or sign due to progression of the underlying disease, etc., the investigator or sub-investigator may confirm that the subject has agreed to participate in the study and obtain the subject's signed and sealed or signed written informed consent from the witness. In this case, the relationship between the witness and the subject, as well as a record of the consent, should also be stored.

A witness is a person who is independent of the conduct of the clinical trial, who is unfairly unaffected by persons involved in the clinical trial, and who attends the informed consent process when the subject is unable to read the consent form, etc.

2） Withdrawal of consent

When a subject participating in this clinical trial requests withdrawal of consent, the principal investigator, etc., should withdraw consent accordingly and record this fact in the medical record.

3） Revision of explanatory documents and informed consent forms (forms) and obtaining reconsent

If the information that may affect the subject's decision is obtained after the commencement of the clinical trial and it is deemed necessary to revise the informed consent form, the investigator or sub-investigator will immediately inform the subject of such information, confirm the subject's willingness to continue participation in the clinical trial, and record the information in the medical record. On the basis of this information, the principal investigator shall revise the explanatory documents and informed consent form and submit the revised explanatory documents and informed consent form to the Institutional Review Board. After obtaining the approval of the Institutional Review Board, the principal investigator, etc., explains it to the subject and obtains written reconsent.

4） Obtaining consent for the transition to the Continuous treatment period

If the subject wishes to continue treatment, written informed consent will be obtained from the subject or from the witness between Week 12 of treatment and the initiation of the Continuous treatment period.

5) Informed consent obtained from a nearby medical institution in which the patient receives the investigational product

If it becomes difficult to attend the hospital as an outpatient, the investigational product administrator may administer the drug at a nearby medical institution. In such cases, written informed consent will be obtained from the subject on the basis of the "Procedures for Delegation of Study Treatment." Consent for self-administration at the subject's home will be obtained by the subject or family.

6) On the subject's health status, such as the reason for progression of the primary disease during the treatment or continuation period

For subjects who wish to self-administer the investigational product during the Continuous treatment period, on the basis of the "Procedures for self-administering the investigational product," written informed consent is obtained from the subject or from the family.

8. ASSIGNMENT OF DRUGS, SUBJECT REGISTRATION, BLINDING

8.1. Randomization of Investigational drug

The investigational product assignment manager will confirm the indistinguishability of the packaging appearance of the investigational product (small box [2 vials] and large box [64 vials]) and subsequently randomly assign the investigational product to the E0302 - 50 mg group and the placebo group on the basis of the investigational product assignment code (key code) prepared by him/her. At this time, the allocation Supervisor of the Investigational Drug will randomly withdraw the investigational drug for storage from each group.

8.2. Method of Assigning Subjects to Treatment Group

8.2.1. Procedures for Subject Registration and Investigational drug Assignment

The investigator or sub-investigator will enroll subjects according to the following procedures:

1) The investigator or sub-investigator will assign a subject identification code to subjects who have provided written consent to participate in the study and record it in the E0302 Subject Screening List and Registry.
2) On the basis of the investigation items at the initiation of the observation period (see 9.2.1.1, Week 0 (at the initiation of the observation period)), the principal investigator or sub-investigator will confirm the eligibility of the subjects and enter the necessary items on the case registration system on the web to determine eligibility. The results of the eligibility evaluation for the observation

registration will be obtained from the case registration system, and eligible patients will be eligible for the observation period. Patients who are ineligible will be ineligible for the observation period.

3) The investigator or sub-investigator will review subject eligibility on the basis of the investigation items at the completion of the observation period (see 9.2.1.2, Week 12 (at the completion of the observation period)) after the completion of the 12-week observation period (initial day of the observation period is Day 0 and Day 84), subsequently enter the necessary items in the case registration system on the web to determine eligibility. Patients eligible for treatment enrollment will be eligible for treatment if indicated by the case registration system. Treatment eligibility will be determined by dynamic allocation (see 8.2.2 Assignment Procedures) and numbered. Patients who are ineligible will be ineligible for treatment.
4) On the basis of the evaluation results obtained from the case registration system, the investigator or sub-investigator will initiate treatment on Day 0 and by Day 3.
5) The investigator or sub-investigator will promptly enter the required information in the case registration system on the website after confirming the discontinuation and completion of treatment for withdrawals, treatment discontinuations, and patients who have completed the Treatment period, respectively.

Patients with no disease progression during the observation period (patients with no change in the total ALSFRS-R score during the observation period or patients with a change in the total ALSFRS-R score of ≥-3) will not be eligible for the observation period. However, if the subject wishes to participate in the study again, he/she will be permitted to participate again only once after obtaining consent in writing. The procedure shall be repeated from 1) in this section.

8.2.2. Method of Assignment

Randomization is by a central registration method, and in order to avoid selection bias in the participating medical organization, the allocation is carried out by a variant of the minimization method, which is a dynamic allocation method. In order of subject enrollment, the investigational product will be assigned as an allocation adjustment factor (minimization factor), taking into account the balance and overall balance between treatment groups within the study site, disease type (bulbar, upper limb, and lower limb onset types), severity at the completion of the observation period of ALS (1, 2 degrees), time from the initial onset to the initiation of the observation period (≤9 months, ≥12 months), %FVC at the completion of the observation period (≤90%, ≥90%), and history of edaravone administration (presence or absence, presence) as the allocation adjustment factor (minimization factor).

8.2.3. Confirmation of Assignment Status

The Medical Statistical Advisor receives reports from the registration center on the status of assignment (subject characteristics by treatment group) masked by the treatment group name as appropriate and, if there is an imbalance in the allocation adjustment factor (minimization factor), recommends a change in the method of assignment to the coordinating investigator and the registration center along with the response measures.

The procedures for enrolling subjects are shown in Figure 8-1.

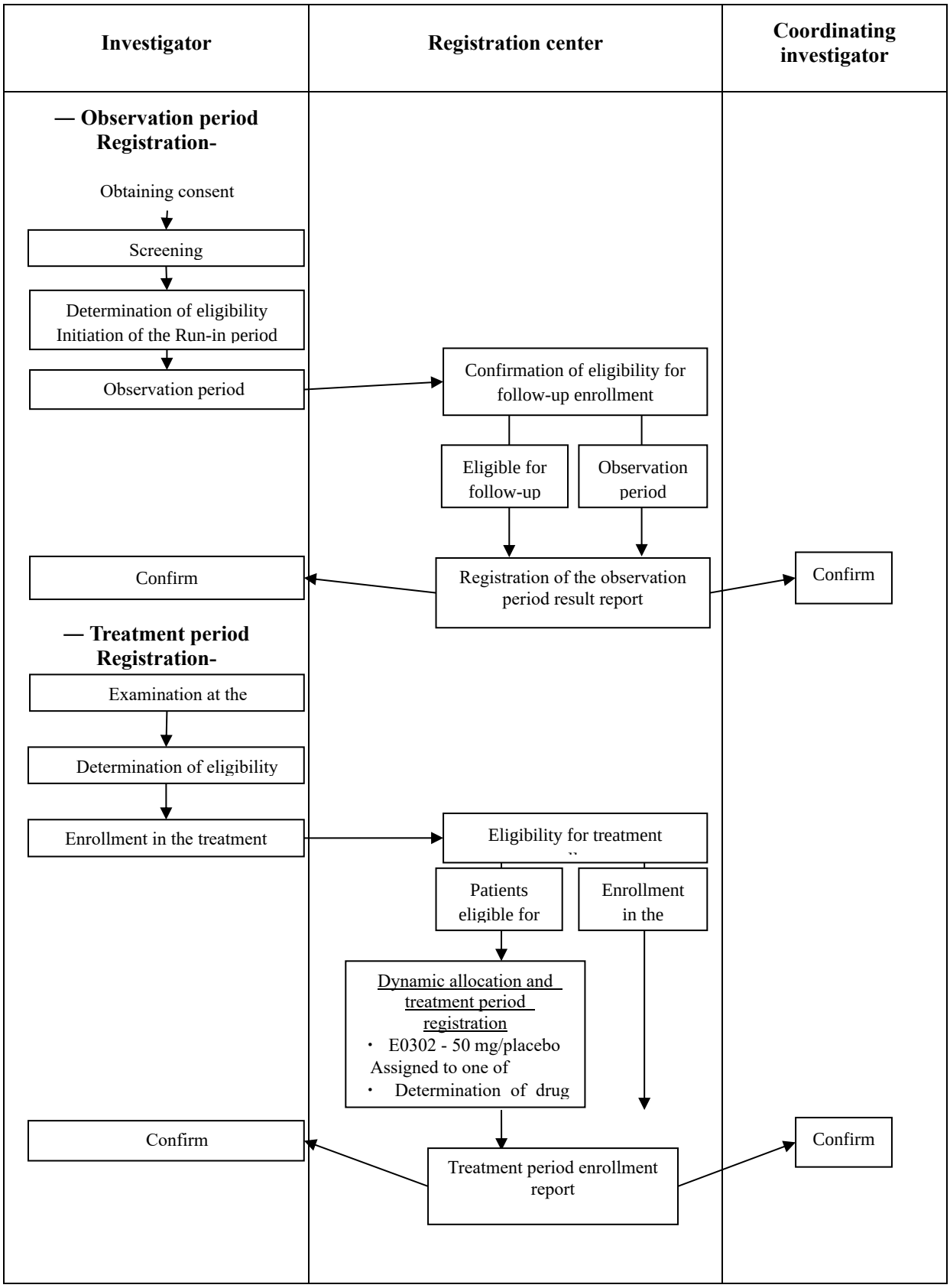

Investigator
Registration center
Coordinating investigator
— Observation period Registration-
Obtaining consent
Screening
Determination of eligibility
Initiation of the Run-in period
Observation period
Confirmation of eligibility for follow-up enrollment
Eligible for follow-up
Observation period
Confirm
Registration of the observation period result report
Confirm
— Treatment period Registration-
Examination at the
Determination of eligibility
Enrollment in the treatment
Eligibility for treatment
Patients eligible for
Enrollment in the
Dynamic allocation and treatment period registration
・ E0302 - 50 mg/placebo
Assigned to one of
・ Determination of drug
Confirm
Treatment period enrollment report
Confirm

Figure 81 Subject Enrollment Procedures1

8.3. Blinding

8.3.1. Confirmation of Indistinguishability

The allocation Supervisor of the Investigational Drug confirms the indistinguishability of the packaging appearance at the time of allocation of the investigational drug and as early as possible from the completion of recall of the investigational drug to expiration of the expiration date for each lot.

8.3.2. Key Code and Emergency Key Code

The allocation Supervisor of the Investigational Drug immediately seals the key code after randomization of the administration group and stores it until disclosure. In addition, after randomization of the administration groups, the investigational product assignment manager seals the emergency key codes prepared to ensure the safety of subjects and stores them until disclosure. At the time of unblinding, the allocation Supervisor of the Investigational Drug ensures that any emergency key codes, other than those unlocked to ensure subject safety, are unopened. The emergency key code other than that unblinded for ensuring the safety of the subject is not unblinded (refer to "12.6 Breaking of the Emergency Key Code" for the procedures for disclosing the emergency key code).

8.3.3. Confirmation of Seal Status after Retrieval of Investigational drugs

The Supervisor of the Investigational Drug shall confirm that unused investigational drugs are sealed, the seal used investigational drugs, and return them to the coordinating investigator.

8.3.4. Securing Blindness of the Investigational drug and Urine Color

- In principle, the clinical trial will be conducted at participating medical organizations through a system that includes at least one efficacy assessor, ALSFRS-R assessor, safety assessor, and investigational drug administrator (see 10.1 "Evaluator and Investigational drug Administrator").
- The efficacy assessor, ALSFRS-R assessor, safety assessor, and investigational drug administrator will not ask the subject regarding the color of the urine. If information regarding the color of urine is obtained, this information shall not be shared with other persons involved in the clinical trial.
- The investigational drug administrator should prepare and administer the investigational product in accordance with the "Procedures for Administration of E0302 Investigational Product" to ensure that the color of the investigational product is not visible to the subject and his/her family. Information regarding the color of the investigational product should not be shared with other persons involved in the clinical trial.
- The investigational drug administrator shall not share information regarding the color of the investigational product with other persons involved in the clinical trial.

8.4. Opening of Emergency Key Code

After locking the database up to Week 16 of treatment for all subjects, the coordinating investigator requests the investigational product assignment manager to break the code.

9. STUDY SCHEDULE

9.1. Study Schedule

The study schedule is shown in Table 9-1.

Table 9-1 Study Schedule

Timing		Observation period		Treatment period (double-blinded)			Continuous treatment period		Discontinuation (Treatment period/ Continuous treatment period)
		Week 0 Initiation	12 weeks (end)	Week 0 (Initial day of administration*1)	Four weeks	Eight weeks	16 weeks (Completed/Discontinued)	Every 12 weeks (Completed/Discontinued)	
Acceptable range (weeks)		—	±1	—	±1	±1	±2	±2	±2*14
Tests, observations, and assessments	Obtainment of written informed consent	●					●*2		
	Patient Characteristics	●	●						
	Registration of the observation period	●							
	Enrollment and assignment during the Treatment period		●						
	Diagnosis (including electromyography and nerve conduction studies)	●*11	●						
	Event occurrence*3			←	―	―	―	―	→
	ALSFRS-R*12	●	●		●	●	●	●	●
	%FVC	●	●			●	●		
	MMTs; handgrip strength testing; Norris scale; ALSAQ-40		●*4			●	●		
	Blood homocysteine (intensive		●				●		
	Clinical Laboratory Tests (Hematology, Biochemistry, Urine)	●*5	●*6		●*7		●*7	●	●
	12-lead ECG	●	●*8	●* 8, 9			●*9, *10	●	
	Vital signs	●	●		●	●	●	●	●
	Administration of the investigational drug			With E0302 - 50 mg/dose or placebo Intramuscular injection twice weekly*13			E0302 - 50 mg/dose Intramuscular injection twice weekly*13		
	Treatment conditions for investigational drug			←	―	―	―	―	→
	Tracheostomy status			←	―	―	―	―	→
	Concomitant medication/treatment *12	←	―	―	―	―	―	―	→
	Adverse events*3	←	―	―	―	―	―	―	→

*1 Should be conducted from Day 0 (Allocation date) to Day3. *2 Obtained informed consent from Week 12 to Week 16. *3 Occurrence of the Event and Adverse event are required to be reported from the last administration date to 28 days later. *4 Conducted in the eligible patients for the treatment period. *5 Measurement of serum Vitamin B12 level are conducted. *6 Women only take a pregnancy test. *7 Conducted before administration of the investigational product. *8 QT assessments are conducted before administration of the investigational product if the first administration is on the allocation date. *9 QT assessments are conducted twice (before administration and 2 hours later after administration). *10 Conducted from Week 8 to the last administration date of Week 15. *11 The results of electromyography and nerve conduction studies conducted in the other medical institutions allowed to be evaluated. *12 Among the patients enrolled in the treatment phase, ALSFRS-R evaluation and investigation of concomitant medication and concomitant therapy will be conducted as much as possible until Week 16 of the treatment phase for the discontinued patients except for the untreated patients (except for those who refuse to continue participation in the study or withdraw consent). *13 For twice-weekly administration of the study drug, the drug will be administered twice during a 7-day period starting from the start date of the treatment/continuation period. The interval between doses should be at least one day, and two doses (4 vials) on the same day is not allowed. ※14 Performed after last administration of the investigational drug and within ±2 weeks from the discontinuation date.

1066

9.2. Investigation/Evaluation Items and Procedures in the Three Period

The study consists of three periods: the Observation period, the Treatment period, and the Continuous treatment period.

9.2.1. Observation Period

The observation period is defined as 12 weeks from the observation period Week 0 (at the initiation of the observation period) to the observation period Week 12 (at the completion of the observation period).

9.2.1.1. Observation period Week 0 (Initiation of the observation period)

For subjects for whom written consent to participate in the study is obtained, tests and observations necessary to determine eligibility will be performed. The investigator or sub-investigator will perform examinations and observations required for qualification at the initiation of the observation period.

At Week 0 of the observation period, the following examinations and evaluations will be performed to evaluate subject eligibility on the basis of the fact that the subject characteristics and inclusion criteria (1) to (4), (6), and (7) are met, and the exclusion criteria (1) to (14) are not met. If ineligible, the appropriate items will be identified. The required items, such as the qualification results, will be entered into the case registration system, and results will be obtained.

(1) Patient characteristics at the initiation of the observation period

1) Subject identification code
2) Date of acquisition of consent
3) Sex
4) Date of birth
5) Diagnosis

・ Diagnosis according to the updated Awaji criteria (see 4.1 Diagnostic Criteria)
・ Diagnosis according to El Escorial revised Airlie House diagnostic criteria (see 4.1 Diagnostic criteria)
(Electromyography and nerve conduction studies may be performed within one year prior to the initiation of the observation period. Electromyography and nerve conduction studies may be evaluated using the results of tests conducted at other medical institutions.)

6) History of the present illness

・ Timing of onset of ALS, type of onset (familial ALS, sporadic ALS), initial presentation (upper extremity, lower extremity type,
Spherical type), and the severity of ALS (see Criteria for Severity of 4.2 ALS)
・ Availability of tracheostomy, non-invasive respiratory support devices
・ Presence or absence of SOD1 mutations (only subjects with previous SOD1 genetic testing)
・ History of administration of edaravone more than four weeks prior to enrollment in the observation period (including reasons for switching)

7) Complications

Diseased at the initiation of the observation period

8) Medical history

・ Drug allergy or severe allergic disease (anaphylactic shock, etc.)
・ Poliomyelitis and neurodegenerative diseases other than ALS (which may affect the onset and pathology of ALS)
Disorders thought to be

9) Concomitant medication/treatment
10) Other

・ Whether the patient is pregnant or possibly pregnant (verbally confirmed: female subjects only)
・ Whether the woman has consented to contraception, including her partner
・ Whether the subject has participated in another clinical trial within 12 weeks prior to the initiation of the observation period

・ Use of riluzole

(2) Efficacy endpoint

1） ALSFRS-R

2） %FVC

(3) Safety endpoint

1） Laboratory tests (including hematology, biochemistry, and vitamin B12 determinations, urinalysis)

2） 12-lead ECG

3） Vital signs (systolic/diastolic blood pressure, pulse rate)

4） Adverse event

9.2.1.2. Observation period Week 12 (Completion of the observation period)

At Week 12 of the observation period, subjects will be assessed for eligibility on the basis of examinations of the following examination and evaluation items, conforming to the subject characteristics and inclusion criteria (3) and (5) to (7), and not conflicting with the exclusion criteria (1)-(4), (7)-(11) and (13)-(14). If ineligible, identify the appropriate items. Enter the required items such as qualification results in the case registration system, and obtain the results and drug numbers.

(1) Patient characteristics at the completion of the observation period

1） Diagnosis

・ Diagnosis according to the updated Awaji criteria (see 4.1 Diagnostic Criteria)

・ Diagnosis according to El Escorial revised Airlie House diagnostic criteria (see 4.1 Diagnostic criteria)

2） History of the present illness

・ Severity of ALS (see Criteria for Severity of 4.2 ALS)

・ Availability of tracheostomy, non-invasive respiratory support devices

3） Concomitant medication/treatment

4） Height and weight

(2) Efficacy endpoint

1） ALSFRS-R

2） %FVC

3） MMT (performed only eligible for treatment)

4） Grip strength test (performed only in eligible patients for treatment)

5） Norris scale (performed only for eligible patients in the treatment period)

6） ALSAQ 40 (performed only in eligible patients for treatment)

7） The concentration of homocysteine in the blood

(3) Safety endpoint

1） Laboratory tests (hematology, biochemistry, urinalysis, pregnancy tests [only premenopausal female subjects except those undergoing sterilization undergo pregnancy tests])

2） 12-lead ECG

3） Vital signs (systolic/diastolic blood pressure, pulse rate)

4） Adverse event

(4) Use of riluzole

9.2.2. Treatment Period (Week 0, 4, 8, 16, and Discontinuation of Study)

The Treatment period is defined as the Treatment period from Week 0 (initial day of administration) to Week 16 of the Treatment period. The following evaluations and investigations on the efficacy and safety will be conducted. However, if discontinuation due to "death" occurs, only information on the occurrence of the event should be checked. Furthermore, %FVC measurement after tracheostomy is not required.

9.2.2.1. Week 0 of the Treatment period

(1) Safety endpoint

1) 12-lead ECG
2) Adverse event

(2) Use of riluzole
(3) Tracheostomy status
(4) Treatment compliance with the investigational product (day of administration, proportion of injections administered at the time of administration, presence or absence of drug holidays, washout period, withdrawal from pharmacology, discontinuation, and reasons for discontinuation)
(5) Concomitant medication/treatment
(6) Discontinuation
Presence or absence of discontinuation, date of discontinuation, and reason for discontinuation

9.2.2.2. Week 4, 8, 16, and Discontinuation of Study

(1) Efficacy endpoint
1) Name of event (daily use of non-invasive respiratory support device, wearing of invasive respiratory support device or death), date of event
2) ALSFRS-R
3) %FVC (performed at 8 and 16 weeks of treatment)
4) MMT (performed at 8 and 16 weeks of treatment)
5) Grip strength test (performed at 8 and 16 weeks of treatment)
6) Norris scale (administered at 8 and 16 weeks of treatment)
7) ALSAQ 40 (performed at 8 and 16 weeks of treatment)
8) The concentration of homocysteine in the blood (only at 16 weeks of treatment)

(2) Safety endpoint
1) Laboratory tests (hematology, biochemistry, and urinalysis) (performed at Weeks 4 and 16, or at the time of discontinuation)
2) 12-Lead ECG (performed once between Week 8 of treatment and the last day of treatment at Week 15 or at the time of discontinuation)
3) Vital signs (systolic/diastolic blood pressure, pulse rate)
4) Adverse event

(3) Use of riluzole
(7) Tracheostomy status
(8) Treatment compliance with the investigational product (day of administration, proportion of injections administered at the time of administration, presence or absence of drug holidays, washout period, withdrawal from pharmacology, discontinuation, and reasons for discontinuation)
(9) Concomitant drug/concomitant therapy (Patients who have experienced an event within 28 days of the last day of administration of the investigational product [the last day of administration of the investigational product is day 0] are to be recorded on the Case Report Form for concomitant treatment until the day of the event)
(10) Discontinuation
Presence or absence of discontinuation, date of discontinuation, and reason for discontinuation

9.2.3. Continuous Treatment Period（Every 12 weeks, Completion or Discontinuation of Study）

The Continuous treatment period is defined as the period from the evaluation at Week 16 of the Treatment period (end date of the Treatment period) to the completion of the Continuous treatment period. The following evaluation/investigation items related to efficacy and safety will be implemented. However, if discontinuation due to "death" occurs, only information on the occurrence of the event should be checked. Furthermore, %FVC measurement after tracheostomy is not required.

(1) Efficacy endpoint
1) Name of event (daily use of non-invasive respiratory support device, wearing of invasive respiratory support device or death), date of the event, and cause of onset
2) ALSFRS-R

(2) Safety endpoint
1) Laboratory tests (hematology, biochemistry, urinalysis)
2) 12-lead ECG
3) Vital signs (systolic/diastolic blood pressure, pulse rate)
4) Adverse event
(3) Tracheostomy status
(4) The status of administration of the investigational drug (judgment of the propriety of self-administration by the principal investigator, etc. {[if applicable] the status of coordination of administration of the investigational drug, including whether it is administered by the subject or by a family member}, the date of administration, the rate of injection administration, the presence or absence of drug holidays, the duration of drug holidays, the absence or absence of pharmacology, the presence or absence of discontinuation, and the reason for discontinuation).
(5) Concomitant medication/treatment
(Discontinuation cases in which an event occurs within 28 days of the last day of administration of the investigational product [the last day of administration of the investigational product is day 0] will be recorded on the Case Report Form concomitant treatment until the day of the event.)
(6) Discontinuation
Presence or absence of discontinuation, date of discontinuation, and reason for discontinuation

10. EVALUATION

On the basis of the statement in this section, efficacy, ALSFRS, and safety evaluators independently conduct evaluations and surveys until Week 16 of the Treatment period. Independent evaluation is not required during the Continuous treatment period, and all evaluations, investigations, and administration of the investigational product are permitted by the investigator and others.

10.1. Person Injecting the Investigational drug and Person Conducting Evaluation

10.1.1. Person in Charge of Efficacy Evaluation

1) Efficacy assessor requirements (it is necessary to meet all of the following)
- The principal investigator or the sub-investigator
- Neurologists (regardless of their department if they have experience with ALS treatment other than neurologists)
- Physicians other than safety assessors
- Physician other than the investigational drug administrator

2) Role of the efficacy assessor

Efficacy variables will be evaluated at the initiation of the observation period, at the completion of the observation period, at Weeks 4, 8, 16 of the Treatment period, or at the time of discontinuation. The efficacy assessor will evaluate the results of the following endpoints:
- Event occurrence
- %FVC
- MMT (only eligible for treatment)
- Grip strength test (performed only in eligible patients for treatment)
- Norris scale (performed only for eligible patients in the treatment period)
- ALSAQ 40 (only in eligible patients for treatment)

10.1.2. Person in Charge of the ALSFRS-R Evaluation

1) Requirements for ALSFRS-R assessors (it is required to meet all of the following)
- The principal investigator, the sub-investigator, or the clinical research coordinator other than the investigational drug administrator shall be the physician or nurse. However, it may be performed by the efficacy assessor.
- ALSFRS-R assessors need to learn in advance using training materials.

2) Roles of ALSFRS-R assessors

ALSFRS-R will be evaluated at the initiation of the observation period, at the completion of the observation period, at Weeks 4, 8, and 16 of the Treatment period, or at the time of discontinuation.

10.1.3. Person in Charge of the Safety Evaluation

1) Requirements of the safety assessor (it is necessary to meet all of the following)
- The principal investigator or the sub-investigator
- Neurologist
- Physicians other than the efficacy assessors
- Physician other than the investigational drug administrator

2) Role of the safety assessor

Symptoms and signs, etc. will be checked by the subject's medical examination (interview, visual inspection, auscultation, etc.) at the initiation of the observation period, at the completion of the observation period, at Weeks 4, 8, 16 of the Treatment period, or at the time of discontinuation, and safety evaluations will be conducted. In addition, instruct the investigational drug administrator to administer the investigational drug. In addition, the efficacy assessor will be informed of the subject's condition, which will need to be worn for all days of non-invasive respiratory support, wear invasive respiratory support devices, or assess the time to death.

If the safety assessor becomes aware of the color of the urine, the information will not be shared with other persons involved in the clinical trial.

10.1.4. Person Injecting the Investigational drug

1) Requirements for subjects who received the investigational product (need to meet all of the following)
- Investigator, sub-investigator, or study collaborator
- Physicians or nurses other than efficacy, ALSFRS-R, and safety assessors

- When administered in-hospital:

Those who can appropriately administer the investigational product in the hospital and prepare the in-house E0302 Administration Record Form and report it to the safety assessor.

- When administered out-of-hospital:

A person who can appropriately administer the investigational product at a patient's home or nearby medical institution and prepare an E0302 treatment record form for external use and report it to the safety assessor.

2) Role of the person receiving the investigational product

In principle, the investigational drug administrator will prepare and administer the investigational product twice weekly in accordance with "5.5 Administration of the investigational product" according to the instructions for administration by the safety assessor.

The investigational drug administrator shall prepare the "In-house E0302 Administration Record Form" when administering the treatment in-house, and the "External E0302 Administration Record Form" when administering the treatment at the subject's home or nearby medical institution, and report to the safety assessor (see "5.5.2 Preparation and Administration Methods").

In order to ensure blinding, the investigational drug administrator should pay attention to the following points:

- Prepare and administer the investigational product in accordance with the "Procedures for Administration of E0302 Investigational Product" and ensure that the color of the investigational product is not visible to the subjects and their families during the Treatment period.
- Information regarding the investigational product and color of urine will not be shared with other study personnel during the Treatment period.
- Place used vials in a small box.
- Dispose of used syringes (needles) appropriately.

1329
1330

10.2. Items and Method of Efficacy Evaluation

10.2.1. ALSFRS-R

The status of activities of daily living and respiratory function will be investigated, the ALSFRS-R (see Table 10-1)[18] will be evaluated, and scores recorded in the Case Report Form in accordance with the ALSFRS-R Assessment Guide. Whenever possible, the same evaluator will evaluate the ALSFRS-R of the same subject.

Table 101 ALSFRS-R10-1

1. Speech 4: Normal speech processes 3: Detectable speech disturbance 2: Intelligible with repeating 1: Speech combined with nonvocal communication 0: Loss of useful speech	6. Dressing and hygiene 4: Normal function 3: Independent and complete self-care with effort or decreased efficiency 2: Intermittent assistance or substitute methods 1: Needs attendant for self-care 0: Total dependence
2. Salivation 4: Normal 3: Slight but definite excess of saliva in mouth; may have nighttime drooling 2: Moderately excessive saliva; may have minimal drooling (during the day) 1: Marked excess of saliva with some drooling 0: Marked drooling; requires constant tissue or handkerchief	7. Turning in bed and adjusting bed clothes 4: Normal 3: Somewhat slow and clumsy, but no help needed 2: Can turn alone or adjust sheets, but with great difficulty 1: Can initiate, but not turn or adjust sheets alone 0: Helpless
3. Swallowing 4: Normal eating habits 3: Early eating problems – occasional choking 2: Dietary consistency changes 1: Needs supplement tube feeding 0: NPO (exclusively parenteral or enteral feeding)	8. Walking 4: Normal 3: Early ambulation difficulties 2: Walks with assistance 1: Nonambulatory functional movement 0: No purposeful leg movement
4. Handwriting 4: Normal 3: Slow or sloppy: all words are legible 2: Not all words are legible 1: Able to grip pen, but unable to write 0: Unable to grip pen	9. Climbing stairs 4: Normal 3: Slow 2: Mild unsteadiness or fatigue 1: Needs assistance 0: Cannot do
5a. Cutting food and handling utensils (patients without gastrostomy)? 4: Normal 3: Somewhat slow and clumsy, but no help needed 2: Can cut most foods, although clumsy and slow; some help needed 1: Food must be cut by someone but can still feed slowly 0: Needs to be fed 5b. Cutting food and handling utensils (alternate scale for patients with gastrostomy)? 4: Normal 3: Clumsy but able to perform all manipulations independently 2: Some help needed with closures and fasteners 1: Provides minimal assistance to the caregiver 0: Unable to perform any aspect of the task	10. Dyspnea 4: None 3: Occurs when walking 2: Occurs with one or more of the following: eating, bathing, dressing (ADL) 1: Occurs at rest, difficulty breathing when either sitting or lying 0: Significant difficulty, considering using mechanical respiratory support 11. Orthopnea 4: None 3: Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows 2: Needs extra pillows in order to sleep (more than two) 1: Can only sleep sitting up 0: Unable to sleep 12. Respiratory insufficiency 4: None 3: Intermittent use of BiPAP 2: Continuous use of BiPAP during the night 1: Continuous use of BiPAP during the night and day 0: Continuous use of BiPAP during the night and day

10.2.2. Event Occurrence

Events are defined as "wearing a non-invasive respiratory assist device all day," "wearing an invasive respiratory assistance device," or "death" due to progression of the primary disease, and their occurrence will be investigated. For "death," however, all events are regarded as events regardless of the cause. When an event occurs, the term of the event and the date of occurrence of the event is recorded in the Case Report Form. The date of the event is the date on which the non-invasive respiratory support device is

worn at night (at least 22 hours), the date on which the invasive respiratory support device is initiated, or the date of death. Even in cases of discontinuation, if an event occurs within 28 days of the last day of administration of the investigational product (the last day of administration of the investigational product is day 0) in the relevant subject, the term of the event, the date of occurrence of the event, and concomitant treatment until the date of occurrence of the event are recorded in the Case Report Form.

In addition, in this clinical trial, "wearing a non-invasive respiratory support device on the whole day" or "wearing an invasive respiratory support device" caused by accidents, accidental complications, etc. will not be handled as an event, and the details of the event, the date of occurrence, and the reason for judgment that the event was not due to progression of the underlying disease will be recorded in the Case Report Form.

When wearing a non-invasive respiratory support device is initiated, in principle, the investigational drug administrator will investigate the total wearing time twice weekly on the day prior to administration and enter the E0302 Administration Record Form for in-house use or the E0302 Administration Record Form for out-of-hospital use. In addition, the efficacy evaluator will understand the status of the subject's respiratory compromise on the basis of information obtained from the safety assessor and the investigational drug administrator and will respond according to the subject's condition. Whenever possible, the same assessor will assess the occurrence of events in the same subject.

10.2.3. %FVC

The %FVC will be measured, and the measurement date, measurement position (standing and sitting position), and measurement results will be recorded in the Case Report Form. The measurement position may be either the standing position or the sitting position, but as far as possible, the same evaluator shall measure it in the same measurement position.

10.2.4. The concentration of Homocysteine in the Blood

The concentration of homocysteine in the blood will be measured centrally by SRL Medisearch Inc., an external laboratory. The results will not be disclosed to the investigators etc., of the participating medical organization until disclosure.

10.2.5. MMT

On the basis of the MMT Examination Sheet (Appendix 5), the MRC score will be used to assess the strength of the neck anteflexion, shoulder abduction, elbow flexion, wrist dorsiflexion, hip flexion, and ankle dorsiflexion (total of 11 muscles), and the score for each muscle strength will be reported on the date of assessment.

10.2.6. Grip Test

Measurements are made using a grip dynamometer. As a rule, two measurements will be taken alternately, and the highest values for each evaluation date and right and left should be reported.

10.2.7. Norris Scale

On the basis of the Norris Scale Worksheet (Appendix 6), the Limb Symptom Scale (21 items) and the Spherical Symptom Scale (13 items) are rated on a 4-point scale, and the date of assessment and scores for each item are reported. In addition, the same evaluator shall perform the test on the same subject as much as possible.

10.2.8. ALSAQ-40

On the basis of the ALSAQ 40 Questionnaire (Appendix 7), the results of the questionnaires administered by the subjects or their families will be reported.

10.3. Items and Method of Safety Evaluation

10.3.1. Clinical Laboratory Test

Laboratory tests will be performed for the following:

Measurement:

- Hematology: WBC, RBC, Hb, Hct, platelet count

- Blood chemistry studies: total protein, total bilirubin, AST (GOT), ALT (GPT), γ-GTP, Al-P, LDH, CK, BUN, creatinine, albumin, total cholesterol, triglycerides, Na, K, Cl, vitamin B12 (at the initiation of the observation period only)
- Urinalysis: Urine sugar, urinary protein, urinary urobilinogen, pregnancy test (only for premenopausal women except those who were sterilized at the completion of the observation period)

In addition, when administering the investigational drug on the day of blood and urine collection, blood and urine are collected prior to administration of the investigational drug.

10.3.2. Electrocardiogram (ECG)

At rest, a 12-lead ECG will be measured, and the measurement date and results (presence or absence of abnormalities and, during the Treatment period, measurements of QT assessment: heart rate, RR interval, PR interval, QRS duration, QT interval, QTcB, and QTcF) will be recorded in the CRF.

During the treatment period, ECG measurements will be performed twice at 1-minute intervals prior to and 2 hours (±1 hour) after dosing, during the first infusion, and between Week 8 (±1 week) and Week 15, the last injection (or discontinuation). As far as possible, this ECG measurement should be performed at the same time period, and the patient will be fasted for at least 8 hours prior to the administration on the day of the visit. In addition, the ECG results during the Treatment period will be sent to the Study Coordinating Office. The ECG should be interpreted by a blinded cardiologist. The results of the reading will be forwarded to the investigator etc., as a report. Corrected intervals to be reported by the cardiologist to the principal investigator, etc., shall be QTcB and QTcF.

Electrocardiogram measurements at the time of discontinuation during the Treatment period are to be performed once, regardless of whether a meal is used. There is also no need to report to a cardiologist.

10.3.3. Vital signs

Blood pressure (systolic and diastolic) and pulse rate will be measured, and the date of measurement and test results will be recorded in the Case Report Form.

10.3.4. Adverse Events

Symptoms and signs related to the safety of subjects that occur after obtaining informed consent will be examined (interview, inspection, auscultation, etc.) and, if any unfavorable observation tended symptoms and signs are observed, recorded in the Adverse Events section in the Case Report Form. However, the incidence of serious adverse events will be investigated up to 28 days after completion (discontinuation) of administration.

Follow-up will generally be conducted until the prognosis of symptoms and signs is known. The time of completion of follow-up will depend on the medical judgment of the safety assessor.

In this clinical investigation, events judged by the safety assessor as aggravation of symptoms associated with progression of the primary disease (including tracheostomy, the appendix of a non-invasive respiratory support device, the appendix of an invasive respiratory support device, appearance or aggravation of subjective symptoms related to decreased respiratory function, and gastrostomy, excluding death) will not be handled as adverse events.

In principle, subjects or their families will be instructed to inform them in advance when they visit other departments or hospitals from the time of acquisition of consent to completion or at the time of discontinuation. The investigator or sub-investigator will check whether the subject has been examined by another department or another medical institution. When a subject visits another department or other hospital, the details of treatment will be checked with the attending physician of another department or other hospital, and information such as adverse events and concomitant treatment will be recorded on the Case Report Form.

10.4. Status of Riluzole Medication

The daily dose, the initial day of administration, and the date of completion of administration of riluzole (when the Treatment period is completed, the date or "continued after completion") will be examined and recorded in the Case Report Form for the status of use of riluzole. If the daily dose of riluzole is changed,

the date of the change, the daily dose after the change, and the reason for the change should be recorded.

10.5. Status of Tracheotomy

The status of implementation of tracheostomy (presence or absence of tracheostomy and date of tracheostomy) will be recorded in the Case Report Form.

10.6. Status of Investigational drug Administration

The drug number and date of administration will be recorded on the Case Report Form on the basis of the Hospital E0302 Administration Record Form and the External E0302 Administration Record Form described by the investigational drug administrator or the E0302 Self-Administration Implementation Sheet written by the subject or family. Two vials will be used at the time of one dose administration. If the administration is not possible due to the subject's condition, the date and details will be recorded in the Case Report Form.

In the event of a drug holiday, the initiation and completion days of the drug holiday are recorded in the Case Report Form.

10.7. Concomitant Treatment

Subjects will be instructed to comply with regulations prohibiting concomitant treatment and restrictions. Combination therapy will also be investigated on the basis of the following:

1) Concomitant medication

For all drugs used during the period from acquisition of consent to completion or discontinuation, the following items will be investigated and recorded in the Case Report Form. When treatment is administered at other departments or other hospitals, the details of the prescription will be checked with the attending physician from other departments or other hospitals and recorded on the Case Report Form. However, physiological saline, infusion, etc., are not required to be recorded on the Case Report Form.

Name of the drug, daily dose, the initial day of administration (if initiated before obtaining informed consent, "continued from before obtaining informed consent"), date of completion of administration (if continued after completion of treatment, "continued after completion of treatment"), route of administration, and reasons for administration (underlying disease, complications, adverse events, and others).

For riluzole, a restricted concomitant drug, the daily dose, the initial day of administration, and the date of completion of administration (when the Treatment period is completed, the date or "continued after completion") will be investigated and recorded in the Case Report Form. If the daily dose of riluzole is changed, the date of the change, the daily dose after the change, and the reason for the change should be recorded.

2) Combination Therapy

For all treatments administered at the time of informed consent, completion, or discontinuation, the following items will be investigated and recorded in the Case Report Form. In the event of treatment at other departments or other hospitals, the details shall be checked with the attending physician of other departments or other hospitals and recorded on the Case Report Form.

Name of therapy, the reason for treatment, date of initiation of treatment (date of continuation prior to the obtainment of informed consent or date of completion of treatment in the case of commencement of administration or later), date of completion of treatment (date of completion of treatment or continuation after completion of treatment in the case of completion of treatment)

Rehabilitation and nutritional management are investigated as follows.

・ Rehabilitation

The presence or absence of rehabilitation will be investigated from the initiation to the completion or discontinuation of the observation period. If rehabilitation has been performed, the name of therapy for rehabilitation (motor function [upper extremity, lower extremity], respiratory function, and frequency, etc.), date of treatment initiation, and date of treatment completion (date or "continued after completion" if treatment is completed) will be recorded in the Case Report Form.

・ Nutritional management

The presence or absence of any treatment related to nutritional management will be investigated from the initiation to the completion or discontinuation of the observation period. If any measures related to nutritional management are implemented, the details (e.g., nasogastric tube feeding, IVH, or PEG) will be investigated, and in the case of nasogastric tube feeding and IVH, the initiation and completion dates will be recorded in the Case Report Form, and in the case of PEG, the date of implementation will be recorded in the Case Report Form.

10.8. Premature Termination or Suspension

The presence or absence of discontinuation, the date of discontinuation, and the reason thereof are recorded on the Case Report Form. If a subject is withdrawn from the study due to pregnancy, the effects on the fetus/birth will be followed up to confirm the results.

11. APPROPRIATENESS OF EFFICACY EVALUATION

The appropriateness of the efficacy investigation items established in this study is shown below.

1) Change in ALSFRS-R total score from the date of randomization to 16 weeks of the Treatment period

The ALSFRS-R is a clinical rating scale designed to objectively and quantitatively assess the course of patients with ALS and can clinically assess the impairment of limb motor, bulbar, and respiratory function in patients with ALS.

ALSFRS-R is frequently used as the primary endpoint in recent studies in patients with ALS. Since ALSFRS-R is reliable enough to evaluate the clinical symptoms of ALS and can be used for clinical evaluation[19] in both total score and item-specific score, the change in ALSFRS-R total score was set as the primary endpoint in this study. In addition, the inter-rater variability was considered to be minimized by having each assessor learn in advance.

2) Time to the event (permanent use of a non-invasive respiratory support device, placement of an invasive respiratory support device, or death)

ALS is a life-threatening disease that occurs within 3 to 6 years of onset. In addition, the WFN guidelines state that "change in muscle strength, survival time is the most informative primary endpoint." Therefore, it was considered essential to consider "survival time" in this study as well. Although "survival time" has been evaluated as the time to death or tracheostomy events in conventional clinical studies in ALS patients, the use of non-invasive respiratory support devices as one of the life support devices for ALS patients has become popular in recent years. Therefore, it is considered appropriate to include the use of non-invasive respiratory support devices throughout the day as an event. For this reason, an event was defined as permanent wear of a non-invasive respiratory assist device, placement of an invasive respiratory support device, or death.

3) %FVC

%FVC has been described in the ALS Treatment Guideline 2013[14] as an objective measure of respiratory function in patients with ALS and is suitable for assessing the progression of respiratory dysfunction. Since it is also used as an endpoint in clinical studies of ALS, the change in %FVC was set as one of the secondary endpoints in this study.

4) The concentration of homocysteine in the blood

Homocysteine has been implicated in the pathogenesis of ALS through a variety of putative neurotoxic mechanisms.[19] To investigate the mechanism of action of E0302, it was considered appropriate to measure the concentration of homocysteine in the blood and set it as an endpoint.

5) MMT

Muscle strength can be assessed noninvasively without the use of special equipment and is also

recommended as one of the appropriate strength tests in the ALS clinical trial guidelines.[21] In addition, it was set because it is used in the clinical trial of ALS.

6) Grip strength

Muscle atrophy was set because grip weakness is often the first symptom, and the measurement of grip strength is simple, the examination time is short, and it is safe.

7) Norris scale

This was established as an evaluation scale of the physical function of the ALS patient and considered to be reliable.[22]

8) ALSAQ-40

This was developed as a QOL scale specific to ALS and was established because it has been validated by reliability (reproducibility) and validity of the original version, and also the validity of the Japanese version.[23]

12. ADVERSE EVENTS, SERIOUS ADVERSE EVENTS, AND THEIR REPORTING

12.1. Definition of Adverse Events

12.1.1. Adverse Events

Adverse events include any unfavorable observation of tended disease or its symptoms, signs, or abnormal changes in laboratory values, etc., occurring in a subject after obtaining informed consent, regardless of relation to the investigational product. Events judged by the safety assessor as aggravation of symptoms associated with progression of the primary disease (including tracheostomy, wearing of a non-invasive respiratory support device, the appendix of an invasive respiratory support device, appearance/aggravation of subjective symptoms related to decreased respiratory function, etc. and gastrostomy, excluding death) will not be handled as adverse events.

12.1.2. Adverse Events Associated with Abnormal Change in Laboratory Parameters

Values within the reference range are considered normal, whereas those outside this range are considered abnormal according to the reference values of the study site.

12.1.2.1. An abnormal change in laboratory findings

If laboratory test values are confirmed and "abnormal values" that are outside the reference range are observed, abnormal changes are defined as medical problems determined by the safety assessor in reference to the "Inclusion Criteria for Laboratory Values and Abnormalities in Blood Pressure and Pulse Rate" (Appendix 3). If there are abnormal changes, the event is handled as an adverse event, and the appropriate term of the event is recorded in the Case Report Form after consideration of the clinical symptoms and signs.

The safety assessor will follow the incident until the outcome of the adverse event is known. The time of completion of the follow-up will be determined according to the medical judgment of the safety assessor.

12.1.2.2. Abnormal changes in ECG and vital signs

The safety assessor will check the electrocardiogram and vital signs and refer to the Criteria for Classification of Seriousness of Adverse Drug Reactions (Appendix 4) for abnormal values. Any finding that meets Grade 2 on the ECG after administration is regarded as an abnormal change and is recorded in the Adverse Events section in the Case Report Form. In addition, in reference to the "Inclusion Criteria for Laboratory Tests and Abnormal Blood Pressure and Pulse Rate" (Appendix 3), the safety evaluator judges whether the change in the relevant measurement is clinically problematic. If the change is considered abnormal at completion or discontinuation, it is handled as an adverse event, and the appropriate term of the event is recorded in the Case Report Form, taking into consideration the clinical

symptoms and signs.

12.1.3. Serious Adverse Events

"Serious adverse event" refers to the following:

(1) Death
(2) Life-threatening
Note: "Life-threatening" means that the subject is at risk of dying when the event occurs and may not have resulted in death if the event is more severe.
(3) Need for hospitalization or prolongation of hospitalization
(4) Results in permanent or significant disability/incapacity
(5) Is a congenital anomaly/congenital disability
(6) Other medically important condition *

*: Significant events that are not immediately life-threatening or leading to death or hospitalization but which jeopardize the subject or require treatment to avoid the consequences described in (1)-(5) above are also considered serious.

In addition, the following hospitalizations that do not accompany adverse events are not regarded as "serious adverse events" among "3) those requiring hospitalization or prolongation of the hospitalization period."

- Hospitalization for temporary treatment for reasons other than an adverse event (short stay, respite hospitalization [short stay for nursing leave])
- Hospitalization due to ambulatory difficulties associated with the progression of the underlying disease
- Hospitalization for Training in Introduction of a Non-invasive Respiratory Assist Device

12.2. Evaluation of Adverse Events

For adverse events that occurred, the term of the event, date of onset, severity, seriousness, outcome, date of the outcome, causal relationship with the investigational product, and treatment of the investigational product will be recorded in the Adverse Events section in the Case Report Form. When recording the name of the disease, the symptoms associated with the disease will not be recorded as an adverse event.

12.2.1. Items of Adverse Events

12.2.2. The severity of Adverse Event

The safety assessor will evaluate the severity of the adverse event on a three-point scale (mild, moderate, or severe) on the basis of the following criteria (Table 12-1) and record it on the Case Report Form. The severity of adverse events on the basis of abnormal changes in laboratory values, electrocardiograms, and vital signs will be assessed by the safety evaluator and recorded on the Case Report Form with reference to Notification No. 80 of the Safety Division, Pharmaceutical Affairs Bureau, MHW, entitled "Criteria for Classification of Seriousness of Adverse Drug Reactions" (Appendix 4). The terms "Grade 1" and "Grade 2" and "Grade 3" as specified in Notification No. 80 of the Safety Division, PAB shall correspond to "Mild" and "Moderate" and "Severe," respectively.

Table 121 Criteria for Severity12-1

Classify	Criteria
1. Mild	Degree of discomfort that does not interfere with normal daily activities
2. Moderate	Degree of discomfort that interferes with normal daily activities
3. Severe	Inability to work or become incapacitated to perform normal daily activities

12.2.3. The seriousness of Adverse Event

1: Non-serious 2: Serious

12.2.4. Reasons for Causal Relationship between Adverse event and Investigational drug

The reason for judgment as serious will be selected from among (1) to (6) in "11.1.3 Serious Adverse Events".

12.2.5. The outcome of Adverse Event

1: Disappearance 2: remission (in recovery) 3: unchangeable 4: deterioration 5: sequelae 6: death

12.2.6. Causal Relationship of Adverse Events with Investigational drug

The causal relationship with the investigational product will be determined by the safety assessor with reference to Table 12-2, taking into account the subject's condition, the temporal relationship between administration of the investigational product and the onset, etc. The causal relationship to the investigational product is "2. Adverse events for which a causal relationship could not be ruled out (adverse reactions). If the event is not related, the event should be classified as "1. Not related" and regarded as an adverse event for which a causal relationship can be ruled out. If an adverse event is considered unrelated, the reason for the assessment will be recorded in the Case Report Form.

Table 12-2 Criteria for Evaluation of Causal Relationship to the Investigational Product for Adverse Events

1. Not relevant.	A clinical event, including an abnormal laboratory finding, in which a temporal relationship between the time of onset of the adverse event and the investigational product is unclear, or in which a cause other than the investigational product (e.g., a risk factor that increases the incidence of adverse events in subjects) is identified.
2. Related	Clinical events, including abnormal laboratory values that occur after administration of the investigational product and occur over a time-course related to the investigational product and are unlikely to be caused by disease at the time of onset, other concomitant drugs, or environmental factors, etc.

12.2.7. Action Taken with Investigational drug

1: Continuation 2: Discontinuation 3: Discontinuation (washout period, washout period), 4: After completion of administration, 5: Other (other reasons)

12.3. Response to Adverse Events

If an adverse event occurs after obtaining informed consent, the investigator or sub-investigator will take appropriate measures to treat the adverse event and continue to monitor it as much as possible until improvement or stabilization (for laboratory values within the reference range of the study site or prior to treatment) is achieved, regardless of the causal relationship with the investigational product. However, this does not apply when the safety assessor determines that the adverse event has disappeared from the effect of the investigational product, ensures the safety of the subject, and does not require further follow-up.

The assessment of adverse events will be assessed by the safety assessor according to "12.1 Definitions of Adverse Events" and "12.2 Assessment of Adverse Events". Adverse events that were considered related to the investigational product are regarded as adverse drug reactions.

In addition, if a serious adverse event is observed, the procedure described in "12.6 Disclosure of the Emergency Key Code" should be followed.

12.4. Serious Adverse Events

12.4.1. Reporting of Serious Adverse Events

If a serious adverse event occurs between the day of the last dose of the investigational drug and Day 28, the investigator or sub-investigator will respond as follows. The serious adverse event may be related to the investigational product. However, if a serious adverse event occurred on or after 29 days after the last dose of an investigational drug for each subject, which the safety assessor judged to be related to the study, is observed, the same measures will be taken.

1) The investigator or sub-investigator should provide the head of each medical institution and the coordinating investigator with the primary report (within 24 hours of knowledge: within one working day at the latest), secondary report (within seven days: not mandatory if a full report is included in the primary report), detailed investigation report, and final report.
2) The coordinating investigator will immediately report the reported serious adverse events (within three working days of the knowledge of the investigator etc.) to the principal investigator and the supplier of the other participating medical organization. In addition, if the reported serious adverse event meets the following requirements, the coordinating investigator will report it to the regulatory authority within the specified time frame.

[REQUIREMENTS FOR REPORTING TO REGULATORY AUTHORITY AND REPORTING DEFINITIONS]

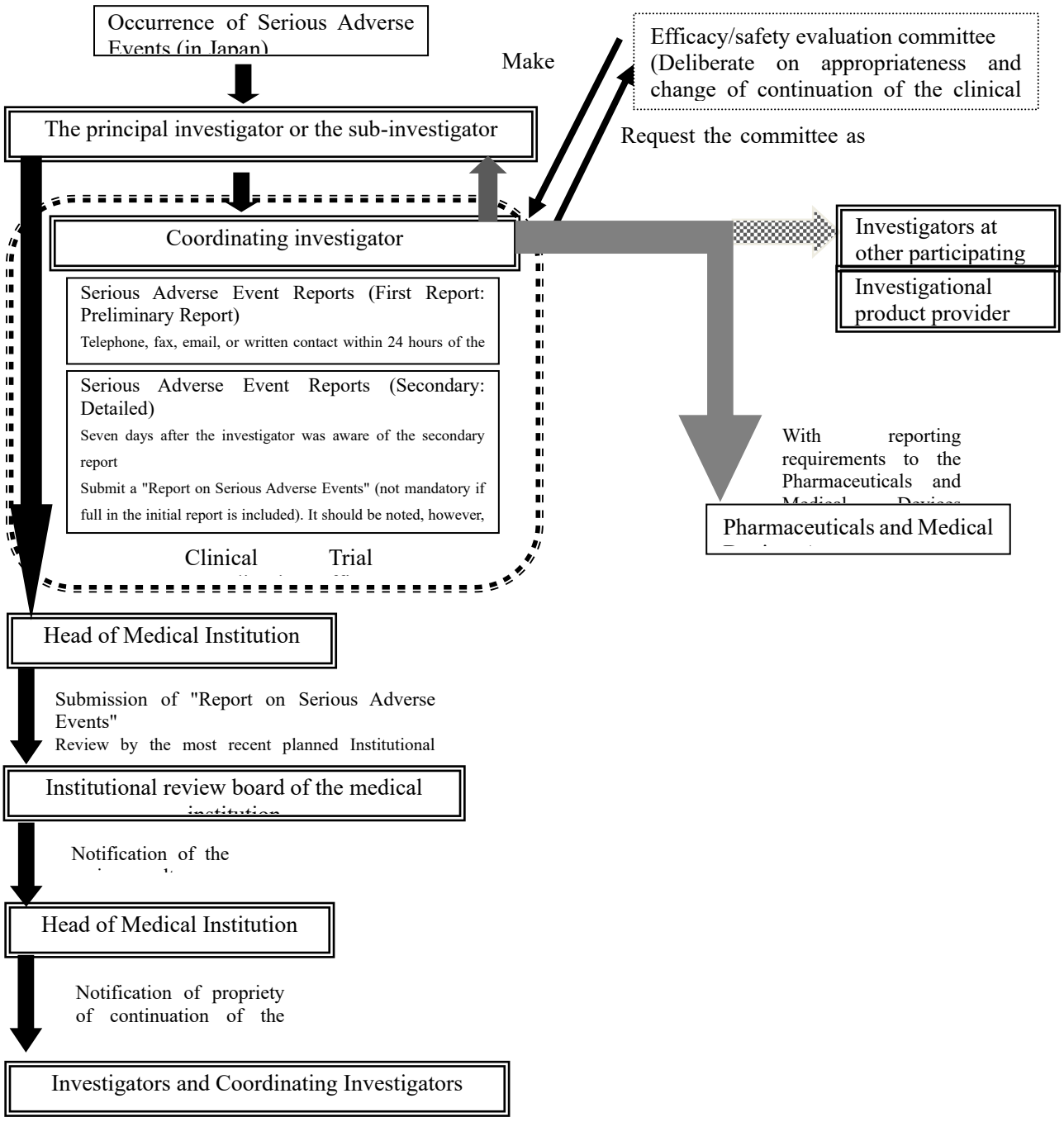
Patients with serious adverse events whose causal relationship to the investigational product cannot be ruled out and who meet the following criteria (1) to (3) are subject to expedited reporting. The coordinating investigator will report the SAE within the following timeframe from the date the investigator becomes aware of the SAE according to its content.

(1) Of the serious adverse events unexpected from the investigator's brochure, "death" and "potentially

fatal" occurred within seven days.
(2) Of the serious adverse events unexpected by the investigator's brochure, within 15 days, except those listed above (1).
(3) Of the serious adverse events expected in the investigator's brochure, "death" and "potentially fatal case" within 15 days.

After consultation with the coordinating investigator, the investigator will seek the opinion of the Efficacy and Safety Assessment Committee on whether to continue the study, etc., as necessary and subsequently decide on the measures to be taken thereafter.

※ In the event of a serious adverse event that may result in death or death related to the investigational product, registration of the patient will be suspended and reviewed by the Efficacy and Safety Evaluation Committee.



*1: The submission deadline, format, etc., shall be in accordance with the provision of Notification No. 0329 of the PFSB and Notification No. 14 of the PFSB.

Figure 12-1 Flow diagram of reporting and response in the event of an SAE.

12.5. Possible Side Effects

No serious adverse events were observed in the Phase I single-dose study (E0302-E044-001) and the Phase I multiple-dose study (E0302-E044-002). In Phase II/III study (E0302-J081-761), cardiac arrest was reported as a serious adverse event for which a causal relationship could not be ruled out. In the Phase III study (E0302-J081-762), urinary stones were reported as serious adverse events for which a causal relationship could not be ruled out. Adverse reactions observed in the Phase I single-dose study, Phase I multiple-dose study, Phase II/III study, and Phase III study are shown in Table 12-2.

Table 122 Adverse Reactions in Clinical Phase I, Phase II/III, and Phase III Studies-2

Study	E0302 Dosage	Administration Pathway.	Side Effects
Investigations of phases 1 Single-dose studies (E0302-E044-001) [Twenty-four Japanese and 24 Caucasians, respectively].	25 mg (n=12)	IM injection	Headache (1 Caucasian)
	50 mg (n=12)	IM injection	Injection site pain (2 events in one Japanese)
	75 mg (n=12)	IM injection	Headache (one Caucasian), nausea (one Caucasian)
	Placebo (n=12)	IM injection	-
Investigations of phases 1 Repeat-dose study (E0302-E044-002) [Eighteen Japanese and 18 Caucasians (7 days)].	25 mg (n=12)	IM injection	-
	50 mg (n=12)	IM injection	Dizziness (one Japanese), vulvovaginal discomfort (one Caucasian), and acneiform dermatitis (one Caucasian)
	Placebo (n=12)	IM injection	-
Phase II/III study (E0302-J081-761) [370 Japanese (182 weeks)].	25 mg (n=124)	IM injection	Injection site induration (1 patient), abnormal liver function (2 patients), leukocytosis (1 patient), increased blood cholesterol (1 patient), increased blood urea nitrogen (1 patient), increased blood alkaline phosphatase (1 patient), sensory disturbance (1 patient), erythema (1 patient), and pruritus (1 patient)
	50 mg (n=123)	IM injection	Cardiac arrest (1 patient), liver disorder (1 patient), folliculitis (1 patient), increased white blood cell count (2 patients), increased platelet count (1 patient), positive urinary protein (1 patient), hypocalcemia (1 patient), urinary stones (1 patient), acne (1 patient), subcutaneous hemorrhage (1 patient), and seborrheic dermatitis (1 patient)
	Placebo (n=123)	IM injection	Injection site pain (2 patients), liver enzyme elevation (1 patient), allergic dermatitis (1 patient), and urticaria (1 patient)
Phase III (E0302-J081-762) [147 Japanese]. Cut-off Data, 31 October 2014*	50 mg (n=147)	IM injection	Supraventricular extrasystole (1 patient), gastroesophageal reflux disease (1 patient), positive urinary protein (3 patients), increased blood bilirubin (1 patient), increased blood urea nitrogen (1 patient), urticaria (1 patient), hypertension (2 patients)

*: The Continuous treatment period was conducted in subjects who completed Study E0302-J081-761. Aggregate data until the last day of the 52-week assessment obtained by the cut-off date as of October 2014. Urinary stones occurred in

one subject as a serious adverse reaction after the last day of Week 52. In addition, no serious side effects were observed in three other clinical studies in ALS.

12.6. Opening of Emergency Key Code

When it is deemed necessary to ensure the safety of subjects, such as when it becomes necessary to know the key code for the investigational product in a medical emergency, the principal investigator can break the emergency key code. The emergency key code-breaking procedure is shown below.

[Emergency key code-breaking procedure]

1) The principal investigator will contact the coordinating investigator if it is deemed necessary to know the details of the investigational product administered to the subject in order to ensure the safety of the subject, such as if a serious adverse event occurs to the subject.
2) The coordinating investigator will interview the necessary items (name of the contacting investigator, drug number, the title of the study subject, and folder-back contact) and request that the allocation manager of the investigational product break the emergency key code of the relevant subject. The investigational product assignment manager will break the emergency key code for the relevant subject and promptly notify the principal investigator of the results thereof by fax.
3) Adverse events that contributed to the request for emergency code unblinding will be handled as serious adverse events. By doing so, the investigator or sub-investigator will promptly report the report to the coordinating investigator and the head of the study site using a predetermined format ("Report on Serious Adverse Events" [Unified Form 12]) and record the reason for requesting emergency key code-breaking in the Case Report Form. The coordinating investigator will record the reason for the request for disclosure, the history of disclosure, and the contact information for the results of disclosure.
4) The coordinating investigator will record the reason why the principal investigator and the coordinating investigator decided that emergency key code-breaking is necessary, the history of emergency key code-breaking, and the contact information for the results of disclosure.

12.7. Opening of Emergency Key Code on the Request of Regulatory Authority

On the basis of the SAEs reported to the regulatory authorities during the study, the regulatory authority may decide that unblinding is necessary to ensure the safety of subjects. If disclosure is requested by the coordinating investigator, emergency key codes may be used to break the code. The emergency key code-breaking procedure is shown below.

[Emergency key code-breaking procedure]

1) On the basis of the serious adverse events reported by the coordinating investigator during the study, the regulatory authority determines that it is necessary to know the allocation results (test drug or placebo) of the investigational product administered to the subject to ensure the safety of the subject. If disclosure is instructed to the coordinating investigator, the coordinating investigator will check the required items (name of the physician, name of the study problem, drug number, and folder-back contact) and request the allocation manager of the investigational product to break the emergency key code of the relevant subject.
2) The investigational product assignment manager will promptly report the results of disclosure to the coordinating investigator.
3) The coordinating investigator will promptly report the results of disclosure to the regulatory authority.
4) When breaking the emergency key code, the coordinating investigator will inform the principal investigator of the details of disclosure and the results of disclosure.
5) The coordinating investigator will record the reason for the regulatory authority's decision that emergency key code-breaking is necessary, the history of emergency key code-breaking, and the contact information for the results of disclosure.

13. DISCONTINUATION OF INDIVIDUAL SUBJECTS

13.1. Criteria for Discontinuation of Study

In the following cases, the investigator or sub-investigator will discontinue the study.

1) When a subject refuses to continue participating in the clinical trial or withdrew consent.
2) When an adverse event occurs and the principal investigator or the sub-investigator judges, it difficult to continue the clinical trial.
3) If the subject's pregnancy was reported.
4) Subjects were found to be ineligible prior to initiation of treatment.
5) Subjects were found to be ineligible after initiation of treatment.
6) When the emergency key code is unlocked.
7) When the principal investigator or the sub-investigator judges the discontinuation of the clinical trial to be appropriate in terms of efficacy evaluation or safety assurance, etc.
8) Withdrawal of at least 15 days from the last dosing day in the Treatment period
9) In the Continuous treatment period, the drug was withdrawn for at least 29 days from the administration day immediately prior to administration.
10) Use of prohibited concomitant drugs or therapies during the period from the initiation of the observation period to Week 16 of the Treatment period (refer to "6.2.1 Prohibited Concomitant Drugs/Treatments" and "6.2.3 Concomitant Therapies").
11) When a new dose of riluzole is initiated during the period from the initiation of the observation period to Week 16 of the Treatment period, the daily dose of riluzole is increased, or the daily dose of riluzole is decreased or discontinued, and subsequently, the dose is increased or re-administered.
12) Events (all-day non-invasive breathing device wear, invasive respiratory support device wear or death)

13.2. Procedures for Discontinuation of Study

The investigator or sub-investigator should promptly inform the subject of the termination of the study and provide appropriate medical care and take other necessary measures. The predetermined parameters at the time of discontinuation (the day on which the physician judged the discontinuation) and the reason thereof is recorded in the Case Report Form. The predetermined parameters will be investigated and evaluated after the last dose of the investigational drug and within ±2 weeks from the date of discontinuation.

If a subject is withdrawn from the study due to pregnancy, follow up the effects on the fetus/offspring and report the results to the coordinating investigator and the investigational product provider.

Even in cases of discontinuation, if an event occurs within 28 days of the last day of administration of the investigational product (the last day of administration of the investigational product is considered day 0) (see "10.2.2 Event Occurrence") in the relevant subject, the term of the event, the date of occurrence of the event, and concomitant treatment until the date of occurrence of the event are recorded in the Case Report Form.

Among patients enrolled in the treatment period, ALSFRS-R evaluations and investigations of concomitant drugs/therapies will be performed as much as possible until Week 16 of the Treatment period unless patients discontinued from the Treatment period, excluding patients who have not received treatment unless they refuse to continue participation in the study or have requested to withdraw consent. If ambulatory is difficult, the ALSFRS-R will be assessed by telephone survey, and concomitant medications/therapies will be surveyed in reference to the ALSFRS-R telephone survey flow diagram.

14. COMPLETION, PREMATURE TERMINATION, OR SUSPENSION OF STUDY

14.1. Completion of Study

The principal investigator confirms that the data have been fixed after completion of the observation, examination, and investigation specified in this protocol for all subjects and reports to the head of the

medical institution that the clinical trial has been completed and the summary of the clinical trial results in writing.

The head of the medical institution shall promptly notify the Institutional Review Board of the completion of the study in writing and report the summary of the results of the study on the basis of the report submitted by the principal investigator. This time point is the completion of the study.

14.2. Premature Termination or Suspension of Study

14.2.1. Criteria for Premature Termination or Suspension

The coordinating investigator will discontinue or suspend the study in consultation with the principal investigator in the following cases:

1) Serious adverse events that may lead to death or death considered related to the investigational product in the study (temporary suspension of patient registration)
2) When it is recommended by the Efficacy and Safety Evaluation Committee that the entire clinical trial be discontinued or suspended
3) When the Institutional Review Board recommends discontinuation or suspension
4) Any change in the development policy of the principal investigator or coordinating investigator
5) Recommendations for discontinuation by the regulatory authority
6) Other situations in which part or all of the clinical trial must be discontinued or suspended.

14.2.2. Procedures for Premature Termination or Suspension

If any item falls under "14.2.1 Criteria for Discontinuation or Suspension," the investigator or sub-investigator will immediately notify the subject to that effect to ensure appropriate treatment and follow-up for the subject.

If discontinuation/suspension of the study is decided, the coordinating investigator will immediately report to all investigators and report to the Efficacy and Safety Assessment Committee, the supplier of the investigational drug, and the regulatory authority. The principal investigators etc., shall promptly inform the subjects of the discontinuation and the reasons thereof to ensure the safety of the subjects.

The principal investigator shall report this in writing to the head of the medical institution, the Institutional Review Board, and the related departments of the participating medical organization and shall follow the procedures specified by the relevant participating medical organization.

The investigational product assignment manager confirms that the investigational product recalled from the study site is sealed by the Supervisor of the Investigational Drug and opens the seal to confirm the number of remaining drugs, etc. Subsequently, seal again, and report the confirmation results of the amount of residual drug to the coordinating investigator.

15. PROTOCOL COMPLIANCE, DEVIATION/AMENDMENT, AND REVISION

15.1. Protocol Compliance

The investigator will initiate the study after the protocol has been approved by the Institutional Review Board and authorization from the head of the study site has been obtained. The content of the protocol should be followed in the conduct of the study.

15.2. Protocol Deviation or Amendment

The principal investigator and the sub-investigator must not deviate from or change the protocol without written approval on the basis of the prior review of the Institutional Review Board, except for the following cases.

(1) When it is unavoidable for medical purposes, such as by avoiding emergent hazards to the subjects
(2) When changes are made only to administrative matters related to the clinical trial

In the case of (1) above, the principal investigator must submit the details and reasons of the deviation or change, and if amendment of the protocol is appropriate to the head of the medical institution and the Institutional Review Board as soon as possible to obtain approval and obtain the agreement of the head of the medical institution.

The principal investigator and the sub-investigator will record all actions that have deviated from the protocol and retain records explaining the reasons, etc.

The principal investigator will promptly submit a report to the head of the medical institution and to the Institutional Review Board on any changes that may seriously affect the conduct of the study or increase the risk to subjects.

15.3. Revision of Protocol and Case Report Form

If an amendment to the protocol is required after the initiation of the study, the investigator will review the protocol and revise it after hearing the opinions of the members of the protocol and report the details and reasons for the amendment to the Institutional Review Board of the participating institution. If the revision is deemed critical, it must be reviewed and approved by the investigator's Institutional Review Board. Refer to the "Procedures for Preparation of the Protocol" for the revision procedure.

16. CASE REPORT FORM

16.1. Preparation and Reporting CRF

Data collection on the Case Report Form (CRF) in this study will be conducted using EDC. EDC is operated according to various procedures prepared in accordance with ER/ES guidelines. Case data will be entered directly from the site using a web browser. These data collected by EDC are regarded as the CRF. Preparation of the CRF shall be in accordance with the separately defined CRF Entry Manual.

Changes or amendments to CRFs will be made in accordance with the separately specified Guidance for Changes and Amendments to CRFs.

16.2. Essential Points in Preparing CRF

CRFs will be prepared promptly.

The principal investigator confirms that there are no problems in the record content of the CRF prepared by the sub-investigator or the study collaborator and subsequently electronically signs the record on the EDC.

If there is any discrepancy between the CRF records and the source documents, the investigator will prepare a record explaining the reasons for the discrepancy.

17. DIRECT ACCESS TO SOURCE DOCUMENTS

17.1. Identification of Source Data

The following documents shall be regarded as source documents.

(1) Medical records, nursing records, examination data, imaging film, and data stored in the electronic medical record
(2) Records related to consent and provision of information to subjects
(3) Records related to the administration of the investigational drug
(4) Records, etc. of Clinical Trials Required by the GCP Ordinance

The CRF records themselves will normally be used as source documents for the following items that are not listed in medical records, etc. However, the relevant medical records, etc., shall be regarded as source documents if they are recorded in the medical records, etc.

(1) Items to be confirmed that they meet the inclusion criteria and do not conflict with the exclusion criteria

(2) Diagnosis, severity, seriousness, the reason for judgment as a serious, outcome, date of the outcome, investigational drug of the adverse event
Causal relationship
(3) Findings for laboratory data
(4) Efficacy Evaluations of the Investigational Product (Scores for Variables of ALSFRS-R)
(5) Purpose of coadministration
(6) Reason for combination therapy
(7) Reason for Request for Disclosure of Emergency Key Code
(8) Reasons for discontinuation

17.2. Direct Access to Source Documents by Monitor

17.2.1. Access to Source Documents

The head of the medical institution and the principal investigator must accept investigations by monitors, auditors, the Institutional Review Board, and regulatory authorities appointed by the coordinating investigator and must provide all study-related records, including source data, for direct access (including copies). In addition, the subject agrees to direct access by signing the consent form.

17.2.2. Monitoring

The investigator has ethical, legal, and scientific responsibility for the conduct of the study. The coordinating investigator will appoint a monitor who will provide or obtain up-to-date information on the study by periodic site visits or by telephone, etc., in accordance with the "Procedures for Monitoring" specific to the study and will retain the records.

18. STATISTICAL ANALYSIS METHOD

Analyses will be performed using the analysis software SAS for Windows (release 9. 3 or more recent). The significance level of the test is 15% on both sides to determine the homogeneity between groups, 2.5% on one side to evaluate the significance of the efficacy endpoint, and 5% on the other side. The analysis will be performed using data from the Treatment period only (at the time of application) and analysis during the Continuous treatment period (at the completion of the study). The main methods of analysis on the basis of data from the Treatment period only are described below, but the analysis On the basis of data from the Continuous treatment period and subsequent periods is performed according to the analysis on the basis of data from the Treatment period only.

18.1. Determination of Sample Size

The target sample size for this study was 64 subjects in each group and 128 subjects in both groups. The rationale for the target number of subjects and the history of changes are described below.

1) Analysis method

This is a randomized controlled trial of two groups (placebo and E0302 - 50 mg) with the primary endpoint of change in ALSFRS-R total score from the date of allocation of to 16 weeks of the Treatment period to verify the superiority of E0302 to placebo. In a mixed-effects repeated measures data analysis (MMRM model), the change in ALSFRS-R total score from randomization to Week 16 will be compared between placebo and E0302 - 50 mg and will be considered significant if the lower bound of the 95% confidence interval for the least-squares mean difference is >0.

2) Endpoints and Estimates Used to Calculate Target Sample Size

In Phase II/III study (E0302-J081-761), an estimate of the change in total ALSFRS-R scores was estimated using data from a subset of patients who experience symptoms for ≤one year at the initiation of the observation period and whose total ALSFRS-R scores decreased by 1-2 points during the observation period (12 weeks). The mean ± SD of the change in ALSFRS-R total score at 16 weeks was-3.2 ± 4.0 in the mecobalamin 50 mg group (26 patients in the E0302 – 50 mg group in this study) and-5.8 ± 5.0 in the placebo group (n = 32) (mean difference: -2.6). On the basis of these

results, the target sample size calculation for this study assumed that the ALSFRS-R total score for the E0302 - 50 mg group was-3.2, the ALSFRS-R total score for the placebo group was-5.8, and the E0302 - 50 mg group outperformed the placebo group by Δ (2.6 points).

To account for the variation due to changes in ALS diagnostic criteria and participating centers compared to the Phase II/III study (E0302-J081-761), the larger standard deviation (5.0) in the mecobalamin 50 mg group and the placebo group (in terms of change in ALSFRS-R total score) was set as the common standard deviation across the study.

3) Calculation of target sample size

A minimum of 60 subjects per group will be required to achieve a type I error probability of ≤2.5% and a power of ≥80% in a one-sided test. In addition, in view of withdrawals during the study, the target number of subjects for this study was 64 per group, totaling 128.

18.2. Statistical Analysis Plan

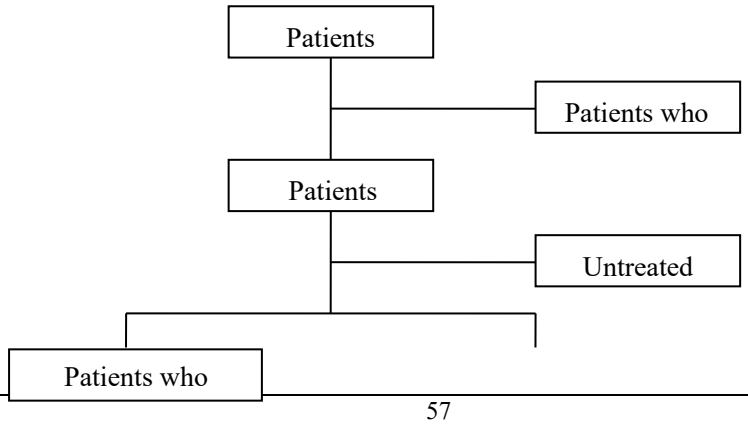
18.2.1. Definition of Analysis Sets

18.2.1.1. Classification of Subjects

The definitions of subjects in this study are shown in Table 18-1 and Figure 18-1.

Table 181 Definitions of Subject Classification-1

Term	Definition
Patients enrolled in the observation period	Subjects who are eligible for the study at the initiation of the observation period, confirmed by the registration center, and enrolled in the observation period.
Discontinuation during the observation period	Subjects who were enrolled in the observation period and were withdrawn from the study without enrollment during the Treatment period
Patients enrolled in the Treatment period	Subjects who are eligible for the study at the completion of the observation period, confirmed by the registration center, and enrolled in the Treatment period.
Untreated cases	Subjects who are enrolled in the Treatment period and have never received the investigational product.
Patients who completed the Treatment period	Subjects who have completed the Treatment period
Patients who discontinued treatment	Subjects who discontinued the study prior to completion of the study Treatment period because they meet the criteria for withdrawal from the study after commencement of study treatment.



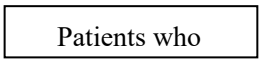


Figure 181 Subject disposition-1

18.2.1.2. Deviations from Study Protocol

Table 18-2 lists the definitions of subjects enrolled in the treatment period who deviate from the protocol. The person responsible for statistical analysis will select subjects meeting these definitions in consultation with the coordinating investigator.

Table 182 Subjects with Protocol Deviations2

Classify	Definition
Deviations prior to enrollment in the Treatment period	Subjects with a protocol deviation prior to enrollment in the Treatment period ・ Ineligible subjects (those who do not meet the inclusion or exclusion criteria) ・ Examples of Deviations Regarding Screening Procedures
Deviations after enrollment in the Treatment period	Subjects with a protocol deviation after enrollment in the Treatment period ・ Deviations in dosage and administration (cumulative injection rate of the investigational product is <70%) ・ Concomitant treatment deviation ・ Examples of Deviations Related to Investigation and Evaluation Procedures ・ Withdrawals not meeting discontinuation criteria ・ Patients who meet the discontinuation criteria but did not discontinue the Treatment period

18.2.1.3. Analytical Handling of Subjects

After consultation with the coordinating investigator, the statistical analysis manager will determine the handling of analysis of all subjects prior to unblinding, on the basis of the definitions of the analysis population described in the following section, after considering the handling of individual subjects for analysis.

18.2.1.4. Efficacy Analysis Set

However, the primary analysis population will be FAS, and PPS will be positioned as a secondary analysis population to confirm consistency with the results obtained from FAS from a sensitivity analysis perspective.

1) FAS

Among patients enrolled in the treatment period, FAS is defined as the set excluding those subjects who meet the following criteria:

・ Subjects who do not meet the primary inclusion criteria (inclusion criteria [1] to [5])
・ Subjects who fall under a GCP violation, such as administration outside the study period or not obtaining informed consent
・ Subjects with no data on the components of the primary endpoint that can be assessed (ALSFRS-R)
・ Subjects who have not received the investigational product

2) PPS

Of the patients enrolled in the treatment period, those who meet the following criteria will be excluded from the population.

・ Subjects who do not meet the primary inclusion criteria (inclusion criteria [1] to [5])
・ Subjects who fall under a GCP violation, such as administration outside the study period or not obtaining informed consent
・ Subjects with no data from Week 8 onward regarding the composition of the primary endpoint that can be assessed (ALSFRS-R)
・ Subjects who met exclusion criteria affecting efficacy assessment (exclusion criteria [1], [8], [14])
・ Subjects who have a cumulative injection rate of <70% of the investigational product prior to the completion or discontinuation date of treatment
・ Subjects who discontinue treatment and participate in the study <8 weeks during the treatment period
・ Subjects who have not received the investigational product

18.2.1.5. Safety Analysis Set

Of the patients enrolled in the treatment period, the safety analysis population (SAS: Safety Analysis Set) excluding those subjects who meet the following criteria will be used.

- Subjects who have not obtained informed consent
- Subjects who do not meet the inclusion criteria for the primary target disease (inclusion criteria [1] to [5])
- Subjects who have not received the investigational product
- Subjects with no evaluable safety data

18.2.1.6. Handling of Data

The handling of data is as follows. The statistical analysis manager should discuss with the coordinating investigator the handling of data for all subjects prior to unblinding and specify the details in the separate analysis plan.

1) Handling of missing data

In order to specify the handling of each item, the items to be supplemented with missing data are described in "5) Handling of data at each evaluation time" or in "Statistical analysis."

2) Handling of Measured Values Out of the Permissible Range Specified

The acceptable ranges for efficacy endpoints (ALSFRS-R, concentration of homocysteine in the blood, %FVC) and safety endpoints (laboratory and vital signs) are one week for Treatment periods 4 and 8 weeks and two weeks for a Treatment period of 16 weeks. For data that deviate from these tolerance ranges, the person responsible for statistical analysis will decide whether or not to use the data in consultation with the coordinating investigator. If more than one data value is within the relevant range, the absolute difference between the specified evaluation date and the number of days is calculated, and the data with the minimum absolute value is used as the data for the evaluation period. If the absolute value is the same, it should be considered separately (by endpoint). Follow-up data will not be handled. ±±

3) Handling of Measured Values for Reasons Other than Tolerance Deviation

Data on the timing of evaluation that may be affected by violations of the investigational product's dosage and administration, concomitant medications, and violations of concomitant therapies, even if the data are within the acceptable range, will be excluded from the analysis of the PPS.

4) Handling of Outliers

Handling of outliers shall be determined by examination prior to unblinding. Depending on the variables, the application of statistical methods that are not significantly influenced by the appropriate transformation or outliers should be considered and defined in the analysis plan.

5) Handling of Data at Each Evaluation Time Period

Except for MMRM analysis, missing data values will not be imputed for all endpoints.

6) Handling of Adverse Events

The number of patients with severe adverse events by severity will be summarized according to the category of severe adverse events ("severe," "moderate," "mild").

7) Handling of Discontinuation Cases

Discontinuations will be censored on the day of discontinuation. However, ALSFRS-R evaluations and investigations of concomitant drugs/therapies will be performed as much as possible until Week 16 of the Treatment period for the patients enrolled in the Treatment period, but not those who discontinued from the Treatment period unless they refuse to continue participation in the study or have requested to withdraw consent. In addition, if an event occurs within 28 days of the last day of administration of the investigational product (the last day of administration of the investigational product is day 0) in a patient who is withdrawn from the study, the efficacy data measured after the event will be analyzed.

18.2.2. Statistical Analysis Method

18.2.2.1. Analysis of subject characteristics

Analysis items: Subject Characteristics
Analysis sets: FAS, PPS, SAS
Analysis method: Continuous variables and ordinal categorical variables will be classified using the Wilcoxon rank-sum test, and continuous and categorical variables (categorical variables) classified by aggregation will be examined for homogeneity between treatment groups using Fisher's exact test.

18.2.2.2. Treatment Status for investigational drug

Analysis items: Treatment status
Analysis sets: FAS, PPS, SAS
Analysis method: Summarize the number and duration of investigational drug administration, cumulative dose, taking into account the rate of administration, presence or absence of drug holidays, reasons for drug holidays, number of drug holidays, days off, discontinuation, and discontinuation of study treatment.
In the Continuous treatment period, the status of administration of the investigational drug in the self-administration is also summarized.

18.2.2.3. Prior and Concomitant Therapies

Analysis items: Pretreatment and concomitant treatment.
Analysis sets: FAS, PPS, SAS
Analysis method: Pretreatment for the underlying disease and combination therapy during the treatment period are summarized. Pharmacotherapy will be summarized according to the WHO-DD dictionary.

18.2.2.4. Efficacy Analysis

18.2.2.4.1. Primary Endpoint

1) Primary analysis

Analysis items: Change in ALSFRS-R total score from the date of randomization to 16 weeks of the Treatment period
Analysis sets: FAS (main analysis), PPS
Analysis method: The change in ALSFRS-R from the date of allocation to each time point is defined as the response variable, and a linear model is fitted with treatment group, time point, minimization factor, the interaction between treatment group, and time point as a fixed effect, ALSFRS-R total score of the date of allocation as a covariate, and covariance structure of error variance as unstructured (unstructured) (MMRM analysis). Significance is considered significant if the lower bound of the 95% least-squares mean confidence interval for the difference in the ALSFRS-R total score at Week 16 of treatment when compared between the placebo and E0302 - 50 mg groups is >0. However, the ALSFRS-R total score of the dates of allocation will be the values measured at the completion of the observation period.

2) Secondary analyses

Analysis items: Change from the date of allocation in the ALSFRS-R total score at Weeks 4 and 8 of the Treatment period
Analysis sets: FAS (main analysis), PPS
Analysis methods: Changes in ALSFRS-R levels at Weeks 4 and 8 of treatment will be tested according to the main analysis. Time-course graphs (including Week 16) of least squares mean ± SEM obtained by MMRM analysis are also plotted for each group. Summary statistics for change from the date of allocation for each group and time point (including worst time) will also be calculated.

18.2.2.4.2. Secondary Endpoint

Analysis Items: Time from the date of randomization to event (permanent use of a non-invasive respiratory support device, wearing an invasive respiratory support device, or death), %FVC change, change in the concentration of homocysteine in the blood, change in MMT total score, change in grip strength (right and left), change in Norris scale total score, and change in ALSAQ 40 total score.

Analysis Sets: FAS (main analysis), PPS

Analysis method : P values for the log-rank test will be calculated for time to event comparisons between groups. Survival curves per group will be generated by the Kaplan–Meier method, and survival rates at each time point will be calculated as appropriate, as well as standard errors according to Greenwood's formula, with corresponding 95% confidence intervals. For changes in the concentration of homocysteine in the blood, change in %FVC, change in MMT total score, change in grip strength (right and left, respectively), change in Norris scale total score, and change in ALSAQ 40 total score at each time point, tests and analyses will be performed according to the primary endpoint.

18.2.2.5. Safety Analysis Set

18.2.2.5.1. Adverse Event

Analysis items: Subject Units, Items (SOCs according to the most recent MedDRA Glossary at Data Fixation, PT) Units Adverse Events/Reactions, Serious Adverse Events, and Adverse Events Leading to Withdrawal

Analysis sets: SAS

Analysis method: The number of patients with adverse events/adverse reactions and the number of them will be tabulated for each treatment group, and the incidence of adverse events/adverse reactions will be calculated. The data will also be tabulated by causality, severity, and severity. Fisher's exact test will be used to compare placebo and E0302 - 50 mg groups for subject-specific AE/ADR rates.

The number of subjects with serious adverse events will be tabulated for each treatment group, and the incidence rate of serious adverse events and their 95% confidence interval (F distribution) will be calculated. The incidence of serious adverse events will be compared between the placebo and E0302 - 50 mg groups using Fisher's exact test. Analyses will also be conducted in which death due to worsening of symptoms associated with the progression of the underlying disease is excluded from serious adverse events.

The number of subjects with adverse events that led to discontinuation will be tabulated for each treatment group, and the incidence rate of adverse events that led to discontinuation and its 95% confidence interval (F distribution) will be calculated.

18.2.2.5.2. Clinical Laboratory Tests

Analysis items: Subject-by-subject, abnormal variation in item-by-item, and laboratory parameters at each evaluation time point.

Analysis sets: SAS

Analysis methods: The incidence of abnormal changes will be calculated for each treatment group, and the placebo group will be compared with the E0302 - 50 mg group using Fisher's exact test.

The change from the end of the observation period (or change) will be calculated for each treatment group, and intragroup comparisons will be made using the Wilcoxon signed-rank test (for one sample). The placebo group will be compared with the E0302 - 50 mg group using the Wilcoxon rank-sum test (two samples).

18.2.2.5.3. Vital signs

Analysis items: Vital Sign Items at Each Evaluation Time

Analysis sets: SAS

Analysis methods: The change from the end of the observation period (or change) will be calculated for each treatment group, and intragroup comparisons will be made using the Wilcoxon signed-rank test (for one sample). The placebo group will be compared with the E0302 - 50 mg group using the Wilcoxon rank-sum test (two samples).

18.2.3. Important Points of Analysis

18.2.3.1. Adjustment Using Covariates

Multiple regression analyses will be used to evaluate the effects of background variables and potentially important prognostic factors that failed to demonstrate homogeneity between treatment groups on the primary endpoint (change in ALSFRS) of the characteristics of subjects (determined prior to unlocking) that may affect the clinical assessment of ALS. Even when the analysis is adjusted for imbalance, the results obtained from the analysis without adjusting for imbalance will be the results of the primary analysis.

The impact on the primary endpoint will also be examined if patients newly initiated on riluzole during the Treatment period or whose daily dose of riluzole was changed are treated as withdrawals. Even when this analysis is performed, the results from an analysis that does not consider such discontinuations will be considered as the primary analysis.

In this study, the diagnostic criteria for ALS (updated Awaji criteria) have been changed from the diagnostic criteria (El Escorial revised Airlie House diagnostic criteria) in Phase II/III study (E0302-J081-761). Therefore, in order to confirm the effect of different diagnostic criteria on the efficacy evaluation, the effect on the primary endpoint will be investigated. The analysis will focus on the subgroups that meet the clinically definite ALS, the clinically probable ALS, and the clinically probable laboratory-supported ALS according to the El Escorial revised Airlie House diagnostic criteria at the completion of the observation period. Even in this analysis, the results from the population eligible according to the updated Awaji criteria are considered to be the primary analysis.

Compared to the Phase II/III study (E0302-J081-761), patients with a history of administration of edaravone within four weeks prior to the observation period were eligible for participation in this study. Therefore, the effect of previous edaravone administration (presence or absence) on the primary endpoint (change in ALSFRS-R) will be examined using multiple regression analyses. Even with this adjusted analysis, the results from the unadjusted analysis will be the primary analysis.

18.2.3.2. Data Monitoring

Data monitoring in this study will include the subject's enrollment status and the occurrence of withdrawals from the study, as appropriate. If it is deemed necessary to revise the duration of enrollment, the duration of treatment or the target number of subjects to be enrolled on the basis of the results of the tabulation in consultation with the Medical Statistical Advisor, the Efficacy and Safety Assessment Committee should be consulted regarding the necessity of amendment of the protocol. If amendments to the protocol are recommended at the meeting of the Efficacy and Safety Assessment Committee, the protocol will be amended according to the recommendations.

The Medical Statistical Advisor will receive a report from the registration center of the allocation status (distribution of subject characteristics by group) with the name of the group, as appropriate. If there is an imbalance in the allocation adjustment factor (minimization factor), the change in the allocation method will be recommended to the coordinating investigator along with the response measures.

18.2.3.3. Interim Analysis

Although an interim analysis will not be conducted in this study, analyses on the basis of data up to the Treatment period (at the time of application) and including the Continuous treatment period (at the completion of this study) will be performed.

18.2.3.4. Recommendations from Independent Data Monitoring Committee

The recommendations made by the Efficacy and Safety Assessment Committee to the sponsor are as follows.

- Discontinuation or continuation due to safety
- Revision of enrollment, Treatment period, or target number of subjects enrolled

Details of the advisory review process and reasons for judgment shall not be disclosed until after disclosure. However, this does not apply to cases in which discontinuation due to safety is recommended and in which the Efficacy and Safety Evaluation Committee determines that discontinuation is warranted from the viewpoint of ensuring the safety of the subjects.

18.2.3.5. Multicenter Study

The target number of subjects in this study is 128 (64 per group). As the number of subjects per site is considered to be insufficient for the assessment of treatment-by-site interaction, independent analyses for each site will not be performed.

18.3. Changes to Analysis Plan and Additional Analysis

When the person responsible for statistical analysis determines that a change or addition to the analysis plan is necessary after the initiation of the study, he/she should consider the appropriateness of the change or addition and the impact on the evaluation of the study. The history of changes or additions to the analysis plan and the results of the analysis will be recorded in the clinical study report.

19. QUALITY CONTROL AND QUALITY ASSURANCE OF CLINICAL STUDY

19.1. Quality Control and Quality Assurance for CRF Data

1) Quality control of data

The person responsible for data management and the person in charge must conduct quality control at each stage of data handling in accordance with separately defined SOPs to ensure the reliability and proper handling of all data related to the clinical trial.

The data management supervisor and persons in charge must conduct data management on the basis of the "Data Management Plan" etc., related to the clinical trial for the procedures until data locking, such as the collection of CRFs, inspection, input, change, and amendment of data, etc.

2) Data quality assurance

The auditor shall confirm that the quality control of the data is appropriately implemented in accordance with the Pharmaceutical GCP Ordinance, Standard Operating Procedures, and this protocol, etc.

19.2. Quality Control

The monitors designated by the coordinating investigator will confirm that the clinical trial is conducted appropriately at the participating medical organization in compliance with the protocol and the GCP Ministerial Ordinance through monitoring, etc. In addition, The source documents and other clinical trial-related records should be directly inspected to ensure the accuracy of the records, such as the CRF.

In the event of any discrepancies with the source documents, consistency of the entries, or logical discrepancies, the investigator should review the validity of the section and correct the CRF as needed.

Monitors will conduct monitoring by site visits, telephone, email, and writing, in accordance with the "Monitoring Procedures" pertaining to the study.

19.3. Quality Assurance

19.3.1. Audit

The auditor appointed by the coordinating investigator evaluates whether the conduct of the study, data preparation, documentation (recording), and reporting is conducted in compliance with the GCP Ordinance and related laws, protocols, and SOPs independently of and separately from normal monitoring and quality control activities of the study.

The auditor shall conduct an audit from the standpoint of a third party as part of the quality assurance activities in accordance with the Audit Plan and the Audit Procedures for the clinical trial concerned.

20. RETENTION OF RECORDS

20.1. Study Institutions

The head of the medical institution shall store the documents to be stored as stipulated in the GCP Ordinance at the participating medical organization.

The storage period of materials is 1. Or 2. Either day is delayed.

① Date of marketing approval for the investigational product (3 years after the date of notification that the discontinuation of development or the results of the clinical trial are not attached to the application for approval)

② Three years after discontinuation or completion of the clinical trial

If the investigator considers that it is unnecessary to store the relevant documents, he/she will notify the head of the medical institution accordingly.

20.2. The organizer of the Institutional Review Board

The person who establishes the Institutional Review Board shall store the documents to be stored as specified by the Ministerial Ordinance on GCP by the Institutional Review Board.

The storage period of materials is 1. Or 2. Either day is delayed.

① Date of marketing approval for the investigational product (3 years after the date of notification that the discontinuation of development or the results of the clinical trial are not attached to the application for approval)

② Three years after discontinuation or completion of the clinical trial

If the investigator considers that it is unnecessary to store the relevant documents, he/she will notify the person who established the Institutional Review Board accordingly.

20.3. Head of Study Institution

The principal investigator shall store the documents to be stored as stipulated in the GCP Ordinance by the principal investigator. The storage location and period of materials shall be in accordance with the "Procedures for Storage of Records."

20.4. Investigator

The coordinating investigator shall store the documents to be stored by the coordinating investigator. The storage location and period of materials shall be in accordance with the "Procedures for Storage of Records."

21. ETHICS

21.1. Institutional Review Board

21.1.1. Review of Study Conduct

The Institutional Review Board will review the protocol, the CRF, the content of the informed consent form to patients, and the appropriateness of the conduct and continuation of the study from an ethical, scientific, and medical perspective.

21.2. Ethical Conduct of Clinical Study

21.2.1. GCP Compliance

In conducting this clinical trial, the protection of human rights of the subjects shall be given utmost priority in accordance with the protocol, "Standards for Implementation of Clinical Trials on Drugs (GCP)" (including amendments dated March 27, 1997) stipulated in Article 14, Paragraphs 3 and 80-2 of the Law Concerning the Security of Quality, Efficacy, and Safety of Drugs and Medical Devices, etc. and in accordance with the Declaration of Helsinki.

21.2.2. Protection of Human Rights

In selecting the subjects, the principal investigator and the sub-investigator will carefully review the appropriateness of requesting their participation in the clinical trial, considering the health status of the subjects, symptoms, age, sex, ability to provide consent, relationship with the principal investigator, and whether they participate in the clinical trial, on the basis of the perspective of protecting human rights and the inclusion and exclusion criteria.

21.2.3. Protection of privacy

When the principal investigator and the sub-investigator provide the CRF, etc., outside the relevant medical institution, they attach a subject identification code and use it. Information (name, address, telephone number, etc.) that can identify the subjects will not be entered by persons outside the medical institution.

When data centers, etc. refer to medical institutions, the identification of subjects will be performed using the subject identification code managed by the principal investigator and the sub-investigator, or the registration number issued by the data center, etc.

When publishing the results of the clinical trial, the investigator or sub-investigator should give full consideration to protecting subjects' names, privacy, diseases, etc., and personal information.

22. STUDY COST AND COMPENSATION/INDEMNITY OF HEALTH INJURY

22.1. Disclosure and Confidentiality

The investigator or sub-investigator shall not have any personal relationships with the supplier of the investigational product used in this clinical trial, such as employment relationships with relatives. These interests in the study will be reviewed and approved by the Institutional Review Board at the participating medical organization in which the principal investigator is employed.

This clinical trial is adopted in "Research Project for Practical Application of Intractable Diseases" of the National Research and Development Corporation of Japan (AMED) and performed in part using public research funds. Investigational products will be provided free of charge by the supplier of the investigational product. The supplier will not influence the results of this study. This trial will also be funded by Eisai Co., Ltd. from April 2020 onwards. The investigator or sub-investigator will not distort his/her professional judgment in the conduct or reporting of the study for financial or other personal benefits. Clinical trials are conducted fairly, and the interests of the manufacturers of products and drugs used in clinical trials are appropriately managed in accordance with the Conflict of Interest Rules of the participating medical organizations.

22.2. Study Cost

Since this clinical trial applies the non-insurance combined medical care expense system, the health insurance of the examinee is adapted under this system except for the cost related to the administration of the investigational drug. The investigational product will be provided free of charge by the supplier of the investigational product, and the expenses related to the administration of the investigational product will

be paid through research funds. In addition, measurements of the concentration of homocysteine in the blood and pregnancy tests are paid through research funds.

When paying the clinical trial cooperative expenses, expenses for reducing the burden, etc., to the subjects, these shall be paid in accordance with the provisions of each participating medical organization.

22.3. Compensation/Indemnity of Study-related Health Injury

1) The head of the medical institution shall provide medical treatment related to the treatment of health damage (adverse reactions, etc.) caused by the subject in connection with the clinical trial and take other necessary measures.
2) Compensation will be paid for death or disability covered by compensation in accordance with the provisions of the contracted clinical trial insurance.
3) No compensation will be provided for any of the health hazards shown in this section if they are denied to be related to this study, if they are caused by the subject's intentional or gross negligence, or if they are caused by a failure of efficacy (the investigational drug was ineffective).

23. TRIAL REGISTRATION, ATTRIBUTION, AND PUBLICATION OF RESULTS

23.1. Clinical Trial Registry

This clinical trial is registered in one of the clinical trial registration systems, Clinical Trial gov., etc., of the Clinical Trial Registration System of the Japan Medical Association, Japic CTI, and UMIN.

23.2. Agreement on Attribution and Publication of Results

The results obtained from this clinical trial shall belong to each participating medical organization. Eisai Co., Ltd., the supplier of investigational products, shall be entitled to use the investigational drug for the purpose of applying for marketing approval.

In addition, the principal investigator and the coordinating investigator will consult with the supplier of the investigational product in advance and select the appropriate international/national conference or peer-reviewed journal for the publication of the study results in various forms (abstracts/posters, presentations, articles, etc.).

24. STUDY ADMINISTRATIVE STRUCTURE

Described in Appendix 1.

25. REFERENCES

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26. APPENDIX

26.1. List of Appendix

Appendix 1: Structure of implementation of the study
Appendix 2: List of drugs prohibited from concomitant use/treatment prohibited from concomitant use
Appendix 3: Inclusion criteria for abnormal laboratory values and blood pressure and pulse rate
Appendix 4: Criteria for Classification of Seriousness of Adverse Drug Reactions
Appendix 5: MMT test sheet
Appendix 6: Norris Scale Worksheet
Appendix 7: ALSAQ 40 Questionnaire

26.2. Appendix 1: Structure of implementation of the study

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26.3. Appendix 2: List of Drugs Prohibited from Concomitant Use/Treatment Prohibited from Concomitant Use

Drugs containing vitamin B12 as the main component	**Date of addition**
Methylcobalamin Cobamamide Hydroxocobalamin Cyanocobalamin	
Drugs considered to have potential efficacy in ALS	
Minocycline Edaravone IGF-I (Insulin-like Growth Factor-1) Dextromethorphan hydrobromide-Quinidine sulfate Tamoxifen Thalidomide Sodium phenylbutyrate Meloxicam Coenzyme Q10 Arimoclomol Perampanel Bosutinib Ropinirole Ibudilast	 30 Oct 2017 6 Nov 2018 6 Nov 2018 24 Apr 2019
Therapy considered to have potential efficacy in ALS	
Hybrid Assistive Limb (Lower Limb Type)	4 Jul 2018

26.4. Appendix 3: Inclusion Criteria for Abnormal Laboratory Values and Blood Pressure and Pulse Rate

The decision will be made by the safety evaluator with reference to the following, taking into account the subject's underlying disease, complications, etc.

Clinical Test items	**Criterion**

Complete Blood Count*1	
White blood cell count	<3,000 /mm^3 The case of increase is not considered an adverse event unless there are special circumstances. Neutropenia (<1,500 /mm^3) and lymphopenia (<800 /mm^3) may be taken as adverse events.
Red blood cell count	Male: <350×10^4 /mm^3, Female: <320×10^4 /mm^3
Hemoglobin	<10 g/dL
Hematocrit value	Male: <35 %, Female: <30 %
Platelet count	Decrease: <7.5×10^4 /mm^3 Increase: ≥60×10^4 /mm^3 with any symptoms, or ≥100×10^4 /mm^3
Blood Biochemical Examination	
AST(GOT) *1 ALT(GPT) *1 γ-GTP*1 ALP*1 LDH*1 CPK*1	>2.5 times of the upper limit of the facility standard value The following examples may be considered as adverse events even if they do not exceed 2.5 times. ・The the contribution of the investigational drug is considered to be significant based on the range of variation. ・An elevated trend is observed during the study, and the patient recovers when the effect of the investigational drug is no longer present.
Albumin*4	<2 g/dL
T-Bil*1 Cre*1 Bun*1	>1.5 times of the upper limit of the facility standad value
Na*1	Decrease: ≤125 mEq/L, Increase: ≥155 mEq/L
K*1	Decrease: ≤3.2 mEq/L, Increase: ≥5.5 mEq/L
Cl*1	Decrease: ≤96 mEq/L, Increase: ≥115 mEq/L
TP	--
TG*4	≥500 mg/dL
T-cho*4	≥400 mg/dL
Urine Examination	
Urine sugar*1 Urine protein*1	Two or more levels of variation (If the qualitative value includes ±, ± shall also be one level.)
Urine urobilinogen	--
Blood Pressure	
Hypertension*2	Systolic blood pressure: ≥140 mmHg or Diastolic blood pressure: ≥90 mmHg
Hypotension*3	Systolic blood pressure: <90 mmHg
Pulse Rate*3	≥100 bpm or <50 bpm

Reference:

*1: Japan Society of Chemotherapy, Antimicrobial Safety Evaluation Standard Review Committee, Final Report “Safety Evaluation Criteria for Antimicrobial Agents.” Japanese Journal of Chemotherapy. JULY 2010; Vol.58, No.4: p484-493.
*2: The Japanese Society of Hypertension. “Hypertension Treatment Guidelines 2014”.
*3: Ministry of Health, Labour and Welfare. “Criteria for Classifying the Severity of Adverse Reactions to Medicinal Products (June 29, 1992) Pharmaceutical Affairs Bill No. 80”.
*4: “Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, Japanese Translation (JCOG ver)”.

26.5. Appendix 4: Criteria for Classification of Seriousness of Adverse Drug Reactions

Criteria for classification of the seriousness of adverse drug reactions follow the statement "Criteria for Classification of Seriousness of Adverse Drug Reactions" prepared by the Ministry of Health, Labour and Welfare.

26.5.1. General remarks

This criterion is based on the classification of the severity of adverse drug reactions into three grades, as follows.

Grade 1: Considered minor side effects.

Grade 2: Not a serious side effect, but not a minor one either.

Grade 3: Considered to be serious side effects. In other words, there is a risk of death or permanent dysfunction to the extent that it interferes with daily life.

26.5.2. Liver

Side effects grade	Grade 1	Grade 2	Grade 3
T-Bil (mg/dL)	≥1.6 and <3.0	≥3.0 and <10	≥10
GOT, GPT (U)	≥1.25×N and <2.5×N ≥50 and <100	≥2.5×N and <12×N ≥100 and <500	≥12×N ≥500
ALP	≥1.25×N and <2.5×N	≥2.5×N and <5×N	≥5×N
γ-GTP	≥1.5×N	-	-
LDH	≥1.5×N	-	-
PT	-	-	≤40%
Other symptoms	-	Icterus Hepatomegaly Right hypochondria Fatty liver	Hemorrhagic diathesis Liver failure Hepatic cirrhosis Hepatophyma Icterus lasting over six months

26.5.3. Kidney

Side effects grade	Grade 1	Grade 2	Grade 3
BUN (mg/dL)	≥1×N and <25	≥25 and <40	≥40
Creatinine (mg/dL)	≥1×N and <2	≥2 and <4	≥4
Proteinuria	1+	2+ or 3+	>3+
Hematuria	Microscopic	Gross	Gross and coagulate
Urine volume	-	≤500ml/24h or oliguria or polyuria	≤500ml/24h or anuria
Serum potassium (mEq/L)		≥5.0 and <5.5	≥5.5
Other symptoms	-	-	Nephrotic syndrome Acute kidney failure Chronic kidney failure Uremia Hydronephrosis

26.5.4. Blood

Side effects grade	Grade 1	Grade 2	Grade 3
Red blood cell	$<350\times10^4$ and $\geq300\times10^4$	$<300\times10^4$ and $\geq250\times10^4$	$<250\times10^4$
Hb (g/dL)	<11 and ≥9.5	<9.5 and ≥8	<8
White blood cell	<4,000 and ≥3,000	<3,000 and ≥2,000	<2,000
Granulocyte	<2,000 and ≥1,500	<1,500 and ≥1,000	<1,000
Platelet	<100,000 and ≥75,000	<75,000 and ≥50,000	<50,000
Hemorrhagic diathesis	Mild bleeding	Moderate bleeding	Severe bleeding
Other symptoms	-	-	Pancytopenia Pure red cell anemia Agranulocytosis

26.5.5. Allergy symptom

Side effects grade	Grade 1	Grade 2	Grade 3
Cutaneous symptom	Local skin rash	Widespread skin rash	Muco‐cutaneo‐ocular syndrome TEN Erythroderma Weber-Christian disease SLE like symptom Scleroderma Pemphigus like symptom
	Photodermatosis, Fixed eruption, Ulceration, Pigmentation, etc		
Constitutional symptom (Fever)	Fever		-
Constitutional symptom (Allergy)	-	-	Shock Anaphylaxis like symptom
	Angioedema		Laryngeal edema
Constitutional symptom (vasculitis)	-	Hypersensitive angiitis	
Local symptom	Arthralgia, Lymphadenopathy		-

26.5.6. Respiratory symptom

Side effects grade	Grade 1	Grade 2	Grade 3
Dyspnea	Short breath Grade 2 by H-J classification	Exertional dyspnea Grade 3 or 4 by H-J classification	Rest dyspnea Grade 5 by H-J classification
Impairment of respiratory rhythm	-	Transient hyperventilation Sleep apnea without symptom or hypoxemia	Respiratory arrest Respiratory depression Continuous hyperventilation Cheyne-Stokes respiration Sleep apnea with symptom or hypoxemia

PaO_2	<70 and ≥60	<60 and ≥50	<50 Decrease of 20 or more in comparison with those before administration
$PaCO_2$	-	-	≥50 (Hypoventilation) ≤30 (Hyperventilation)
%FVC % in 1 second	-	<70 % and ≥50 % <70 % and ≥50 %	≤50 % ≤50 %
Chest X-ray (Consolidation)	-	Less than 1/3 of one lung	More than 1/3 of one lung
Chest X-ray (Interstitial shadow)	-	-	Diffuse interstitial shadow
Chest X-ray (Pleural effusion)	-	Less than 1/3 of one lung	More than 1/3 of one lung
Asthmatic attack	-	Minor asthma attack	Moderate and Severe asthma attack
Hemoptysis	-	Hemosputum	Hemoptysis
Other symptoms	Hiccup, Yawning, Hoarseness, Sneezing, Nasal obstruction, Cough, Sputum, Sore throat, Chest pain	-	ARDS, Interstitial pneumonia, PIE syndrome, Pulmonary cirrhosis, Hypersensitivity pneumonia, Pulmonary edema, Pulmonary embolism, Pulmonary vasculitis, Glossoptosis, Laryngospasm, Glottis edema, Pulmonary hypertension

26.5.7. Gastrointestinal symptom

Side effects grade	Grade 1	Grade 2	Grade 3
Nausea, Vomit	Nausea	Vomit	-
Diarrhea	Loose passage	Watery stool not applicable to Grade 3	Watery stool with dehydration and/or electrolyte abnormalities
Gastrointestinal bleeding	Fecal occult blood (+)	Bloody stool or Hematemesis without shock and anemia (Hb ≤8.0g/dL)	Bloody stool or Hematemesis with shock and anemia (Hb ≤8.0g/dL)
Abnormalities in the oral cavity	Subjective discomfort in the oral cavity	Ulcerative stomatitis	-
	Objective discomfort in the oral cavity with inflammation etc		-
Abnormalities in the esophagus	Subjective discomfort in the esophagus	Objective discomfort in the esophagus with inflammation etc	
Dysphagia	-	Difficulty in	Impossible to swallow

		swallowing	
Abnormalities in the gastrointestinal	Subjective discomfort in the gastrointestinal	-	-
Pain	Stomach or bowel pain not applicable to Grade 2	Excruciating pain	-
Inflammation	Gastritis, Enteritis, Colitis, and Proctitis		
	-	Hemorrhagic colitis, Pseudomembranous colitis	
Ulcer	Erosion	Gastric ulcer, Duodenal ulcer, Hemorrhagic ulcer, Small intestine ulcer, Colon ulcer	Gastrointestinal perforation
Ireus	Constipation		Ileus paralytic
Abnormality in the anal	Subjective discomfort in the anal	-	-
	Objective discomfort in the anal with inflammation etc		-
Disorder in the pancreas	Abnormal value of amylase	Pancreatitis not applicable to Grade 3	Pancreatic necrosis, Hemorrhagic pancreatitis
Other symptoms	Hiccup, Thirstiness, Belch, Pigmentation in the colonic mucous membrane, Meteorism, Flatus, Sulphurous odor, Frequent bowel movement	-	-
	Sialadenitis, Copracrasia		-

26.5.8. Cardiovascular symptom

Side effects grade	Grade 1	Grade 2	Grade 3
Decrease of blood pressure (mmHg)	-	<90 and ≥80	<80
Decrease of blood pressure	Syncope, Orthostatic dizziness, Orthostatic hypotension		Pulselessness
Increase in blood pressure	Increase in blood pressure, Hypertension		-
Cardiovascular disorder	-	-	Shock, Cyanosis, Peripheral circulatory failure
Tachycardia (bpm)	-	≥110 and <130	≥110
Bradycardia (bpm)	-	<50 and ≥40	<40
Arrythmia	Palpitation, Arrhythmia (ECG unmeasured), Supraventricular premature contraction, Ventricular premature contraction (single),	Supraventricular tachycardia, Ventricular premature contraction (double), Bigeminal pulse, Atrial tachycardia, Atrial fibrillation, Paroxysmal	Ventricular premature contraction (more than triple, multifocal), Ventricular tachycardia (more than sextuple), Ventricular fibrillation, Torsades de pointes, Third-degree

	First-degree atrioventricular block	tachycardia, Second-degree atrioventricular block, Atrioventricular dissociation, Sinus pause, Bundle branch block, Nodal rhythm, Ventricular rhythm	atrioventricular block, cardiac arrest, Adams-Stokes syndrome
Abnormal ECG	P-wave disappearance PR, PQ extension	ST increase, ST decrease, T-wave inversion, T-wave leveling, U-wave, QT extension, wide QRS	-
Heart failure like a symptom	60%≥ LVEF >50%, 20≤ pulmonary artery systolic pressure <30, Shortness of breath, Hugh-Jones classification grade 2	Edema (whole body, local), 50%≥ LVEF >40%, 2.5 L/min/m^2≥ Cardiac index, 30≤ pulmonary artery systolic pressure <40, Dyspnea on exertion, Hugh-Jones classification grade 3,4	Congestive heart failure, Right heart failure, Left heart failure, acute heart failure, Cardiac enlargement, 40%> LVEF, 2.2 L/min/m^2≥ Cardiac index, 40< pulmonary artery systolic pressure, Dyspnea at rest, Hugh-Jones classification grade 5
Ischemic heart disease like a symptom	Chest discomfort, Chest agony, Chest tightness	-	Deterioration of angina pectoris, Angina attacks, Myocardial infarction, Myocardial necrosis
	Chest pain, Anginal pain, Myocardial ischemia, Coronary insufficiency		
Impairment of myocardium, pericardium, endocardium	-	Pericarditis, Pericardical effusion, Arteriosclerosis	Myocarditis, Myocardial fibrosis
	Myocardial dysfunction		
Vascular disease	Vascular pain	Vasospasm, Intermittent claudication, Arteriosclerosis	Gangrene, Vasuculitis, Thrombophlebitis, Thrombosis, Thromboembolism
	Raynaud's syndrome		
Other symptoms	Flushing, Feverish, Burning sensation, Hot flash	-	-

26.5.9. Neuropsychiatric symptom

Side effects grade	Grade 1	Grade 2	Grade 3
Psychic activity and behavioral disorder	Subjective mood elevation, mood instability, depressed motivation, the	Objective mood elevation, mood instability, depressed motivation, the	Severe and uncontrollable symptom in grade 2, Continuous delusion, hallucination, delirium,

	decline in intellectual ability, Insomnia	decline in intellectual ability with a behavioral disorder, Insomnia	
Disturbance of consciousness	Subjective disturbance of consciousness	Objective disturbance of consciousness	Severe and continuous symptom in the grade 2
Movement disorder	Mild myalgia and arthralgia, Transient and mild involuntary movement, Subjective abnormal muscle tension, Subjective speech impairment, Decreased reflexes	Objective gait impairment, Objective muscle weakness, Severe and continuous myalgia, and arthralgia, Continuous involuntary movement, Severe abnormal muscle tension, Objective speech impairment, Transient oculomotor disturbance, Hyperreflexia, Hyporeflexia	Severe and continuous symptom in grade 2 requires assistance with daily activities
Convulsion	Subjective symptom	Local convulsion	General convulsion
Sensory impairment	Subjective hearing impairment, visual impairment,	Objective and transient hearing impairment, visual impairment	Irreversible hearing impairment, visual impairment, olfactory impairment, taste disorder, sensory impairment
	Transient olfactory impairment, taste disorder, sensory impairment		
Neuropathy	Transient neuralgia	Continuous neuralgia	Severe and continuous symptom in grade 2 requires assistance with daily activities
Dependency	-	Mild psychodependence with the trend toward increased use	Physical dependence, Withdrawal symptoms
Other symptoms	Yawning, Syncopy, Floating sensation, headache Dizziness, Head pressure sensation, Fatigue, Feeling sick, Lethargy	Difficulty in swallowing, Drooling	Dysphagia, Malignant syndrome, Malignant hyperthermia, Encephalopathy, Cerebral meningitis, Cerebrovascular disease

26.5.10. Metabolic and electrolyte abnormalities

Side effects grade	Grade 1	Grade 2	Grade 3
Abnormality of blood glucose	Casual blood glucose: 120 - 200, Fasting	Casual blood glucose: 201 - 300, Fasting	Casual blood glucose: ≥ 300, Hyperglycemic coma,

	blood glucose: 120 - 140, Postprandal blood clucose: 160 - 200, Blood glucose: 60 - 69	blood glucose: 141 - 200, Postprandial blood glucose: 201 - 300, Blood glucose: 50 – 59, Dizziness, Headache, Hunger sensation, Irritation, Prominent symptoms due to hypoglycemia	Blood glucose: ≤50, Hypoglycemic coma, Convulsion
Metabolic acidosis	pH 7.20 - 7.35	pH 7.15 - 7.20	pH <7.15 Disturbance of consciousness, Decrease of blood pressure, Convulsion, Respiratory failure
Metabolic alkalosis	pH 7.46 - 7.50	pH 7.50 - 7.60	pH ≥7.60 Convulsion, Tetanus, Hypertension, Arrythmia
Abnormality of blood calcium	10.6 - 12.1 8.0 - 8.5	12.1 - 15.0 6.5 - 8.0	≥15, <6.5 Disturbance of consciousness, Tetanus, Decrease of blood pressure, Arrhythmia, Psychiatric symptoms
Abnormality of blood potassium	5.0 - 5.5 3.1 - 3.5	5.5 - 6.0 2.5 - 3.1	≥6.0, <2.5 Arrhythmia, Muscular weakness, Arrhythmia
Abnormality of blood sodium	150 - 155 125 - 135	155 - 160 115 - 125	≥160, <115 Disturbance of consciousness, Convulsion, Psyciatoric symptoms, Pathological reflex

26.6. Appendix 5: MMT test sheet

Manual Muscle Test (MMT) sheet		
To evaluate the muscle strength using the Medical Research Council (MRC) score※ (Neck flexion, shoulder abduction, elbow flexion, wrist flexion, hip flexion, and ankle flexion). ※MRC score 0: Nocontraction 1: Flicker or trace of contraction 2: Active movement, with gravity, eliminated 3: Active movement against gravity 4: Active movement against gravity and resistance 5: Normal power		
Subject number		
Date of examination		
Site of measurement		MRC score
Shoulder abduction	Right	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
	Left	☐0 ☐1 ☐2 ☐3 ☐4 ☐5

Elbow flexion	Right	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
	Left	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
Wrist flexion	Right	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
	Left	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
Hip flexion	Right	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
	Left	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
Ankle flexion	Right	☐0 ☐1 ☐2 ☐3 ☐4 ☐5
	Left	☐0 ☐1 ☐2 ☐3 ☐4 ☐5

26.7. Appendix 6: Norris Scale Worksheet

Modified Norris Scale Worksheet	
Subject number	
Date of examination	
Name of evaluator	

Limb Norris Scale

		Normal (3)		Impaired (2)		Trace (1)		Impossible (0)	
1	Hold up head	☐	Able to hold 60 degrees flexion position	☐	Able to hold 30 degrees flexion position	☐	Able to hold 0 - 30 degrees flexion position	☐	Unable
2	Turn over	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
3	Rise from a supine position to a sitting position	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
4	Write your name	☐	Normal	☐	Able to do it alone by using a pen with effort and time (readable)	☐	Able to do it by using a marker pen with effort and time (readable)	☐	Unable
5	Wear shirts	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
6	Fasten buttons on your shirts	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
7	Wear pants	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
8	Draw a line with a ruler	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it alone but it's not practical, or able to do it with a self-service tool	☐	Unable
9	Grab a fork or spoon	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it alone but it's not practical, or able to do it with a self-service tool	☐	Unable
10	Pour tea from a teapot into a	☐	Normal	☐	Able to do it alone with	☐	Able to do it with assistance	☐	Unable

	bowl and drink it				effort and time		or a self-service tool		
11	Stand up and bow	☐	Normal	☐	Able to do it alone with effort and time	☐	Unable to do it in enough form	☐	Unable
12	Comb your hair	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
13	Brush your teeth	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance or a self-service tool	☐	Unable
14	Lift a book or tray	☐	Normal	☐	Able to lift a light book	☐	Able to lift an empty tray	☐	Unable
15	Lift a pen	☐	Normal	☐	Able to do it alone with effort and time	☐	Unable to do it in enough form	☐	Unable
16	Change the position on your arms	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
17	Go upstairs	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
18	Walk 50 meters	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to walk within 50 meters	☐	Unable
19	Walk alone	☐	Normal	☐	Able to walk everywhere with effort and time	☐	Able to walk in limited places and distances	☐	Unable
20	Walk with assistance	☐	Able to walk without assistance	☐	Able to walk with assistance	☐	Able to walk 1 meter with assistance	☐	Unable
21	Stand up from a sitting position	☐	Normal	☐	Able to do it alone with effort and time	☐	Able to do it with assistance	☐	Unable
Norris Bulbar Scale									
		Normal (3)		Impaired (2)		Trace (1)		Impossible (0)	
1	Breethe out all at once	☐	Normal	☐	Able to do it, but it’s weak	☐	Breath leak into you nose	☐	Unable
2	Whistle	☐	Normal	☐	Able to do it, but it’s weak	☐	Unable to whistle but able to pout	☐	Unable
3	Puff up your cheeks	☐	Normal	☐	Breath leaks out when your cheeks are pressed	☐	Able to close lips but cheeks don't puff out	☐	Unable to close lips
4	Move your jaw	☐	Able to move in any direction	☐	Able to do it, but it’s weak	☐	Able to do it, but it’s extremely weak and slow	☐	Unable
5	Say ”la la la"	☐	Normal	☐	Able to say slowly	☐	Pronunciation is unclear	☐	Unable
6	Stick out your tongue	☐	Normal	☐	Able to stick out your tongue beyond your lips	☐	Able to stick out your tongue to your dentition	☐	Unable to stick out your tongue to your dentition
7	Place your tongue on the	☐	Able to place your tongue on	☐	Able to place your tongue on	☐	Able to place your tongue on	☐	Unable to place your tongue on

	inside of your cheek		the inside of your cheek and contract your tongue strongly		the inside of your cheek, but contraction is weak		the inside of your cheek but no contraction		the inside of your cheek
8	Place your tongue on your maxilla	☐	Able to place your tongue strongly against the maxilla	☐	Able to place and hold your tongue against the maxilla	☐	Able to move your tongue upward	☐	Hardly able to move your tongue
9	Cough	☐	Normal	☐	Able to cough and expectorate, but it's weak	☐	Able to cough but unable to expectorate	☐	Unable
10	Drool	☐	None	☐	Drool while looking down, eating, and talking	☐	Drool even when not looking down, eating and talking, or sometimes need to wipe the drool off	☐	Constantly drool
11	Nasal sound	☐	None	☐	A little	☐	Remarkable	☐	Unable to understand
12	Dysarthria	☐	None	☐	Sometimes unable to understand	☐	Sometimes able to understand	☐	Hardly able to understand
13	Meals	☐	Regular food	☐	Soft food	☐	Minced food	☐	Semifluid food

26.8. Appendix 7: ALSAQ 40 Questionnaire

26.8.1. ALSAQ 40（Japanese version）

ALSAQ 40 (Japanese version) was used in this study.

Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 (ALSAQ 40)	
被験者識別コード	
アンケート実施日	
記載者	☐ 患者 ☐ 家族 (続柄:)
以下の質問は，ここ 2 週間であなたに生じたかもしれない問題について説明したものです。それぞれについてその状況がどれくらいあなたに起こったか，最もよくあてはまる番号にひとつだけ○印をつけて下さい。	次１～２０は，ここ 2 週間であなたに生じたかもしれない問題に

ついて説明したものです。それぞれについてその状況どくいなにこたか，最よあはる号ひつけ。印をけ下さい。

		まったくなかった	ほとんどなかった	ときどきあった	しばしばあった	いつもそうだった／まったくできない
1	たとえば家のまわりなど，短い距離を歩くのがむずかしかったことがある	1	2	3	4	5
2	歩いている途中で，転んだことがある	1	2	3	4	5

3	歩いていて，つまずいたり，よろけたりしたことがある	1	2	3	4	5
4	歩いている途中で，バランスを失ったことがある	1	2	3	4	5
5	歩くことに神経を集中しなければ歩けなかったことがある	1	2	3	4	5
6	歩いていて，へとへとに疲れたことがある	1	2	3	4	5
7	歩いていて，足に痛みを感じたことがある	1	2	3	4	5
8	階段ののぼりおりがむずかしかったことがある	1	2	3	4	5
9	立っているのがむずかしかったことがある	1	2	3	4	5
10	いすから立ち上がるのがむずかしかったことがある	1	2	3	4	5
11	腕や手を動かすのがむずかしかったことがある	1	2	3	4	5
12	寝床で寝がえりをうつのがむずかしかったことがある	1	2	3	4	5
13	ものをひろい上げることがむずかしかったことがある	1	2	3	4	5
14	本や新聞をつかんだり，ページをめくったりすることがむずかしかったことがある	1	2	3	4	5
15	ものをはっきり書くことがむずかしかったことがある	1	2	3	4	5
16	家事をすることがむずかしかったことがある	1	2	3	4	5
17	自分で食事をすることがむずかしかったことがある	1	2	3	4	5
18	髪をとかしたり，歯みがきをすることがむずかしかったことがある	1	2	3	4	5
19	服を着ることがむずかしかったことがある	1	2	3	4	5
20	洗面台で洗うことがむずかしかったことがあ	1	2	3	4	5

	る					
21	飲み込むことがむずかしかったことがある	1	2	3	4	5
22	固形のものを食べることがむずかしかったことがある	1	2	3	4	5
23	液体を飲むことがむずかしかったことがある	1	2	3	4	5
24	会話に参加することがむずかしかったことがある	1	2	3	4	5
25	自分が話したことが理解されにくかったと感じたことがある	1	2	3	4	5
26	話している途中で言葉がはっきりしなくなったり，どもったりしたことがある	1	2	3	4	5
27	非常にゆっくりとしか話せなかったことがある	1	2	3	4	5
28	以前より話さなくなった	1	2	3	4	5
29	思うように話せなくていらいらしたことがある	1	2	3	4	5
30	話すときにまわりを気にしたことがある	1	2	3	4	5
31	さみしいと思ったことがある	1	2	3	4	5
32	退屈だと思ったことがある	1	2	3	4	5
33	マナーと違うことをして，はずかしいと思ったことがある	1	2	3	4	5
34	将来に希望がもてないと思ったことがある	1	2	3	4	5
35	自分は他の人にとって負担になっているのではないかと心配したことがある	1	2	3	4	5
36	自分はなぜ今の生活を続けているのかと思ったことがある	1	2	3	4	5
37	この病気のために腹を立てたことがある	1	2	3	4	5
38	ゆううつな気分になったことがある	1	2	3	4	5
39	将来，この病気によってどのような影響を受	1	2	3	4	5

	けるのか心配になったことがある					
40	自分にはまったく自由がないのではないかと感じたことがある	1	2	3	4	5

26.8.2. ALSAQ 40（English version）

ALSAQ 40 (English version) was created for the English protocol version.

Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 (ALSAQ 40)						
Subject number						
Date of examination						
Describer		☐ Patient ☐ Family (Relationship:)				
The following questions are descriptions of problems you may have had while walking in the last two weeks. For each, please check the number that best describes how often the situation occurred to you.						
		Never	Rarely	Sometimes	Often	Always or cannot do at all
1	I have found it difficult to walk short distances (e.g., around the house).	1	2	3	4	5
2	I have fallen over the whole walking.	1	2	3	4	5
3	I have stumbled or tripped while walking.	1	2	3	4	5
4	I have lost my balance while walking.	1	2	3	4	5
5	I have had to concentrate while waliing.	1	2	3	4	5
6	Walking has tired me out.	1	2	3	4	5
7	I have had pains in my legs while walking.	1	2	3	4	5
8	I have found it difficult to go up and down the stairs.	1	2	3	4	5
9	I have foun it difficult to stand up.	1	2	3	4	5
10	I have found it difficult to get myself up out of chairs.	1	2	3	4	5
11	I have had difficulty using my arms and hands.	1	2	3	4	5
12	I have found turning and moving in bed difficult.	1	2	3	4	5
13	I have found picking things up difficult.	1	2	3	4	5
14	I have hound holding books or newspapers, or turning pages, difficult.	1	2	3	4	5
15	I have had difficulty writing clearly.	1	2	3	4	5
16	I have found it difficult to do jobs around the house.	1	2	3	4	5
17	I have found it difficult to feed myself.	1	2	3	4	5
18	I have had difficulty combing my hair or cleaning my teeth.	1	2	3	4	5
19	I have had difficulty getting dressed.	1	2	3	4	5
20	I have had difficulty washing at the hand	1	2	3	4	5

	basin.					
21	I have had difficulty swallowing.	1	2	3	4	5
22	I have had difficulty eating solid food.	1	2	3	4	5
23	I have found it difficult to drink liquids.	1	2	3	4	5
24	I have found it difficult to participate in conversations.	1	2	3	4	5
25	I have felt that my speech has not been easy to understand.	1	2	3	4	5
26	I have slurred or stutterer while speaking.	1	2	3	4	5
27	I have had to talk very slowly.	1	2	3	4	5
28	I have talked less than I used to do.	1	2	3	4	5
29	I have been frustrated by my speech.	1	2	3	4	5
30	I have felt self-conscious about my speech.	1	2	3	4	5
31	I have felt lonely.	1	2	3	4	5
32	I have been bored.	1	2	3	4	5
33	I have felt embarrassed in social situations.	1	2	3	4	5
34	I have felt hopeless about the future.	1	2	3	4	5
35	I have worried that I am a burden to other people.	1	2	3	4	5
36	I have wondered why I keep going.	1	2	3	4	5
37	I have felt angry because of the disease.	1	2	3	4	5
38	I have felt depressed.	1	2	3	4	5
39	I have worried about how the disease will affect me in the future.	1	2	3	4	5
40	I have felt as if I have no freedom.	1	2	3	4	5

27. DOCUMENT CHANGE HISTORY

Ver 1.1 (2017.10.31)

・**P.10 Overview [Statistical Methods] 1) Efficacy analysis**

Reason for change: Error.

Before: Differences are considered significant if the lower boundary of the 95% least-squares mean confidence interval for the change in ALSFRS-R total score at Week 16 compared between the placebo and E0302-50 mg groups is ≤0.

After: Differences are considered significant if the lower boundary of the 95% least-squares mean confidence interval for the change in ALSFRS-R total scores at Week 16 compared between the placebo and E0302-50 mg groups is ≥0.

・**P.56 18.1 Determination of Sample Size 1) Analysis method**

Reason for change: Error.

Before: In a mixed-effects repeated measures data analysis (MMRM model), the change in ALSFRS-

R total score from randomization to Week 16 will be compared between placebo and E0302 - 50 mg and will be considered significant if the lower bound of the 95% confidence interval for the least-squares mean difference is ≤0.
After: In a mixed-effects repeated measures data analysis (MMRM model), the change in ALSFRS-R total score from randomization to Week 16 will be compared between placebo and E0302 - 50 mg and will be considered significant if the lower bound of the 95% confidence interval for the least-squares mean difference is ≥0.

・**P.56 18.2.2.4.1. Primary Endpoint 1) Primary analysis, Analysis method:**
Reason for change: Error.
Before: The change in ALSFRS-R from the date of allocation to each time point is defined as the response variable, and a linear model is fitted with treatment group, time point, minimization factor, the interaction between treatment group, and time point as a fixed effect, ALSFRS-R total score of the date of allocation as a covariate, and covariance structure of error variance as unstructured (unstructured) (MMRM analysis). Significance is considered significant if the lower bound of the 95% least-squares mean confidence interval for the difference in the ALSFRS-R total score at Week 16 of treatment when compared between the placebo and E0302 - 50 mg groups is <0.
After: The change in ALSFRS-R from the date of allocation to each time point is defined as the response variable, and a linear model is fitted with treatment group, time point, minimization factor, the interaction between treatment group, and time point as a fixed effect, ALSFRS-R total score of the date of allocation as a covariate, and covariance structure of error variance as unstructured (unstructured) (MMRM analysis). Significance is considered significant if the lower bound of the 95% least-squares mean confidence interval for the difference in the ALSFRS-R total score at Week 16 of treatment when compared between the placebo and E0302 - 50 mg groups is >0.

Ver 1.2 (2018.1.24)

・**P.13 2. HISTORY OF DEVELOPMENT (BACKGROUND INFORMATION)**
Reason for change: Correction of descriptions
Before: In addition, 41 patients with ALS received mecobalamin 50 mg versus no mecobalamin in an open, non-randomized, controlled trial.
After: In addition, 41 patients with ALS received mecobalamin 50 mg (repeated IM twice weekly) versus no mecobalamin in an open, non-randomized, controlled trial.

・**P.13 2. HISTORY OF DEVELOPMENT (BACKGROUND INFORMATION)**
Reason for change: Update of the results
Before: On the other hand, in the subgroup of subjects with ALS onset ≤12 months, dose-response prolonged the time to the event and reduced the ALSFRS-R total score.
After: On the other hand, in the subgroup of subjects with ALS onset ≤12 months (48 subjects in the placebo group, 54 subjects in the 25 mg group, and 42 subjects in the 50 mg group), dose-response prolonged the time to the event and reduced the ALSFRS-R total score.

・**P.13 2. HISTORY OF DEVELOPMENT (BACKGROUND INFORMATION)**
Reason for change: Update of the results
Before: In addition, a Phase III clinical study (E0302-J081-762) has been conducted by Eisai Co., Ltd. as an extension study of Phase II/III study (E0302-J081-761) to investigate the safety and efficacy of continuous long-term administration of E0302 - 50 mg in ALS patients. From the results of the 52 weeks assessment by the cut-off point (31 January 2014), there was no particular concern regarding the safety of long-term administration.
After: In addition, a Phase III clinical study (E0302-J081-762) was conducted by Eisai Co., Ltd. as an extension study of Phase II/III study (E0302-J081-761) to investigate the safety and efficacy of continuous long-term administration of E0302 - 50 mg in ALS patients. From the results of the 52

weeks assessment by the cut-off point (31 October 2014), there was no particular concern regarding the safety of long-term administration.

・**P.22 5.3. Storage of Investigational Drug**
Reason for change: Correction of descriptions
Before: The Supervisor of the Investigational Drug shall appropriately manage the investigational drug in accordance with the "Procedures for Handling of the E0302 Investigational Drug" and prepare the Investigational Drug Accountability Record to understand the status of use of the investigational drug and the progress of the study. The investigational product should be stored in a light-resistant container at room temperature.
After: The Supervisor of the Investigational Drug shall appropriately manage the investigational drug in accordance with the "Procedures for Management of the E0302 Investigational Drug" and prepare the Investigational Drug Accountability Record to understand the status of use of the investigational drug and the progress of the study. The investigational product should be stored in a light-resistant container at room temperature.

・**P.22 5.4. Delivery of Investigational Drug to Study Institution**
Reason for change: Correction of descriptions
Before: The Supervisor of the Investigational Drug shall record the status of receipt and export of the investigational drug, the status of use of the investigational drug for each subject, and the recall or disposition of unused investigational drugs in the Investigational Drug Accountability Record. Refer to the " Procedures for Handling of the E0302 Investigational Drug " for details of records related to the management of the investigational product.
After: The Supervisor of the Investigational Drug shall record the status of receipt and export of the investigational drug, the status of use of the investigational drug for each subject, and the recall or disposition of unused investigational drugs in the Investigational Drug Accountability Record. Refer to the " Procedures for Management of the E0302 Investigational Drug " for details of records related to the management of the investigational product.

・**P.25 5.6.1. Subjects who can attend the outpatient clinic**
Reason for change: Correction of descriptions
Before: In this clinical trial, the investigational product manager will appropriately store and manage the investigational product according to the " Procedures for Handling of the E0302 Investigational Drug ". The principal investigator will dispense the investigational product to the investigational product manager. The investigational product manager will dispense the product to the administrator. Subsequently, he/she will administer the drug to the subject.
After: In this clinical trial, the investigational product manager will appropriately store and manage the investigational product according to the " Procedures for Management of the E0302 Investigational Drug ". The principal investigator will dispense the investigational product to the investigational product manager. The investigational product manager will dispense the product to the administrator. Subsequently, he/she will administer the drug to the subject.

・**P.25 5.6.2.1. During the Treatment Period**
Reason for change: Newly created procedures are clearly marked.
Before: In principle, during the mid-Treatment period, the drug will be administered to subjects in outpatient clinics. If ambulatory visits become difficult due to reasons such as the progress of the underlying disease, they will be permitted to be administered by the investigational drug administrator at the subject's home or nearby medical institution. Storage of the investigational product at the subject's home is permitted. The following precautions should be taken when storing the investigational product at the subject's home.
After: In principle, during the mid-Treatment period, the drug will be administered to subjects in

outpatient clinics. If ambulatory visits become difficult due to reasons such as the progress of the underlying disease, they will be permitted to be administered by the investigational drug administrator at the subject's home or nearby medical institution. In addition, refer to the "Procedures for Delegation of Study Treatment." Storage of the investigational product at the subject's home is permitted. The following precautions should be taken when storing the investigational product at the subject's home.

・P.26 6.2.1. Prohibited Concomitant Drugs and Therapy
Reason for change: Correction of descriptions
Before: 1) Edaravone (Appendix 2)
After: 1) Drugs with Possible Efficacy in ALS (Appendix 2)

・P.27 7.2. Obtaining Written Informed Consent 1) Timing and Methods of Initial Informed Consent
Reason for change: Error
Before: If the subject has sufficient capacity to provide informed consent, but it is difficult to sign and seal or sign due to progression of the underlying disease, etc., the investigator or sub-investigator may confirm that the subject has agreed to participate in the study and obtain the subject's signed and sealed or signed written informed consent from the substitute. In this case, the relationship between the substitute and the subject, as well as a record of the consent, should also be stored. The substitute shall be the subject's spouse, custodian, guardian, or another person equivalent to the subject's substitute. He or she shall be a person who, in view of the substance of the lives of both parties and the joint spiritual relationship between them, is likely to be in the subject's best interests.
After: If the subject has sufficient capacity to provide informed consent, but it is difficult to sign and seal or sign due to progression of the underlying disease, etc., the investigator or sub-investigator may confirm that the subject has agreed to participate in the study and obtain the subject's signed and sealed or signed written informed consent from the witness. In this case, the relationship between the witness and the subject, as well as a record of the consent, should also be stored. A witness is a person who is independent of the conduct of the clinical trial, who is unfairly unaffected by persons involved in the clinical trial, and who attends the informed consent process when the subject is unable to read the consent form, etc.

・P.28 7.2. Obtaining Written Informed Consent 4）Obtaining consent for the transition to the Continuous treatment period
Reason for change: Error
Before:
After: If the subject wishes to continue treatment, written informed consent will be obtained from the subject or from the witness between Week 12 of treatment and the initiation of the Continuous treatment period.

・P.28 7.2. Obtaining Written Informed Consent 5) Informed consent obtained from a nearby medical institution in which the patient receives the investigational product
Reason for change: Error
Before: No description.
After: If it becomes difficult to attend the hospital as an outpatient, the investigational product administrator may administer the drug at a nearby medical institution. In such cases, written informed consent will be obtained from the subject on the basis of the “Procedures for Delegation of Study Treatment." Consent for self-administration at the subject's home will be obtained by the subject or family.

・P.28 7.2. Obtaining Written Informed Consent 6) On the subject's health status, such as the

reason for progression of the primary disease during the treatment or continuation period
Reason for change: Error
Before: No description.
After: For subjects who wish to self-administer the investigational product during the Continuous treatment period, on the basis of the “Procedures for self-administering the investigational product," written informed consent is obtained from the subject or from the family.

・**P.35 9.2.1.1. Observation period Week 12 (Completion of the observation period)**
Reason for change: Correction of descriptions
Before:
(1) Patient characteristics at the initiation of the observation period
5）Diagnosis
・ Diagnosis according to the updated Awaji criteria (see 4.1 Diagnostic Criteria)
・ Diagnosis according to El Escorial revised Airlie House diagnostic criteria (see 4.1 Diagnostic criteria)
After:
(1) Patient characteristics at the initiation of the observation period
5）Diagnosis
・ Diagnosis according to the updated Awaji criteria (see 4.1 Diagnostic Criteria)
・ Diagnosis according to El Escorial revised Airlie House diagnostic criteria (see 4.1 Diagnostic criteria)
(Electromyography and nerve conduction studies may be performed within one year prior to the initiation of the observation period. Electromyography and nerve conduction studies may be evaluated using the results of tests conducted at other medical institutions.)

・**P.51 Table 122 Adverse Reactions in Clinical Phase I, Phase II/III, and Phase III Studies**
Reason for change: Completion of Phase III study (E0302-J081-762)
Before:

Study	E0302 Dosage	Administration Pathway.	Side Effects
Investigations of phases 1 Single-dose studies (E0302-E044-001) [Twenty-four Japanese and 24 Caucasians, respectively].	25 mg (n=12)	IM injection	Headache (1 Caucasian)
	50 mg (n=12)	IM injection	Injection site pain (2 events in one Japanese)
	75 mg (n=12)	IM injection	Headache (one Caucasian), nausea (one Caucasian)
	Placebo (n=12)	IM injection	-
Investigations of phases 1 Repeat-dose study (E0302-E044-002) [Eighteen Japanese and 18 Caucasians (7 days)].	25 mg (n=12)	IM injection	-
	50 mg (n=12)	IM injection	Dizziness (one Japanese), vulvovaginal discomfort (one Caucasian), and acneiform dermatitis (one Caucasian)
	Placebo (n=12)	IM injection	-
Phase II/III study (E0302-J081-761) [370 Japanese (182 weeks)].	25 mg (n=124)	IM injection	Injection site induration (1 patient), abnormal liver function (2 patients), leukocytosis (1 patient), increased blood cholesterol (1 patient), increased blood urea nitrogen (1 patient), increased blood alkaline phosphatase (1 patient), sensory

			disturbance (1 patient), erythema (1 patient), and pruritus (1 patient)
	50 mg (n=123)	IM injection	Cardiac arrest (1 patient), liver disorder (1 patient), folliculitis (1 patient), increased white blood cell count (2 patients), increased platelet count (1 patient), positive urinary protein (1 patient), hypocalcemia (1 patient), urinary stones (1 patient), acne (1 patient), subcutaneous hemorrhage (1 patient), and seborrheic dermatitis (1 patient)
	Placebo (n=123)	IM injection	Injection site pain (2 patients), liver enzyme elevation (1 patient), allergic dermatitis (1 patient), and urticaria (1 patient)
Phase III (E0302-J081-762) [120 Japanese]. Cut-off Data, 31 January 2014*	50 mg (n=120)	IM injection	Supraventricular extrasystole (1 patient), gastroesophageal reflux disease (1 patient), positive urinary protein (3 patients), increased blood bilirubin (1 patient), increased blood urea nitrogen (1 patient), urticaria (1 patient), hypertension (2 patients)

*: The Continuous treatment period is ongoing in subjects who completed Study E0302-J081-761. Aggregate data until the last day of the 52-week assessment obtained by the cut-off date as of January 2014. Urinary stones occurred in one subject as a serious adverse reaction after the last day of Week 52. In addition, no serious side effects were observed in three other clinical studies in ALS.

After:

Study	E0302 Dosage	Administration Pathway.	Side Effects
Investigations of phases 1 Single-dose studies (E0302-E044-001) [Twenty-four Japanese and 24 Caucasians, respectively].	25 mg (n=12)	IM injection	Headache (1 Caucasian)
	50 mg (n=12)	IM injection	Injection site pain (2 events in one Japanese)
	75 mg (n=12)	IM injection	Headache (one Caucasian), nausea (one Caucasian)
	Placebo (n=12)	IM injection	-
Investigations of phases 1 Repeat-dose study (E0302-E044-002) [Eighteen Japanese and 18 Caucasians (7 days)].	25 mg (n=12)	IM injection	-
	50 mg (n=12)	IM injection	Dizziness (one Japanese), vulvovaginal discomfort (one Caucasian), and acneiform dermatitis (one Caucasian)
	Placebo (n=12)	IM injection	-
Phase II/III study (E0302-J081-761) [370 Japanese (182 weeks)].	25 mg (n=124)	IM injection	Injection site induration (1 patient), abnormal liver function (2 patients), leukocytosis (1 patient), increased blood cholesterol (1 patient), increased blood urea nitrogen (1 patient), increased blood

			alkaline phosphatase (1 patient), sensory disturbance (1 patient), erythema (1 patient), and pruritus (1 patient)
	50 mg (n=123)	IM injection	Cardiac arrest (1 patient), liver disorder (1 patient), folliculitis (1 patient), increased white blood cell count (2 patients), increased platelet count (1 patient), positive urinary protein (1 patient), hypocalcemia (1 patient), urinary stones (1 patient), acne (1 patient), subcutaneous hemorrhage (1 patient), and seborrheic dermatitis (1 patient)
	Placebo (n=123)	IM injection	Injection site pain (2 patients), liver enzyme elevation (1 patient), allergic dermatitis (1 patient), and urticaria (1 patient)
Phase III (E0302-J081-762) [147 Japanese]. Cut-off Data, 31 October 2014*	50 mg (n=147)	IM injection	Supraventricular extrasystole (1 patient), gastroesophageal reflux disease (1 patient), positive urinary protein (3 patients), increased blood bilirubin (1 patient), increased blood urea nitrogen (1 patient), urticaria (1 patient), hypertension (2 patients)

*: The Continuous treatment period was conducted in subjects who completed Study E0302-J081-761. Aggregate data until the last day of the 52-week assessment obtained by the cut-off date as of October 2014. Urinary stones occurred in one subject as a serious adverse reaction after the last day of Week 52. In addition, no serious side effects were observed in three other clinical studies in ALS.

・**P.52 18.1. Determination of Sample Size**

Reason for change: Error

Before:

2) Endpoints and Estimates Used to Calculate Target Sample Size

In Phase II/III study (E0302-J081-761), an estimate of the change in total ALSFRS-R scores was estimated using data from a subset of patients who experience symptoms for ≤one year at the initiation of the observation period and whose total ALSFRS-R scores decreased by 1-2 points during the observation period (12 weeks). The mean ± SD of the change in ALSFRS-R total score at 16 months was-3.2 ± 4.0 in the mecobalamin 50 mg group (26 patients in the E0302 – 50 mg group in this study) and-5.8 ± 5.0 in the placebo group (n = 32) (mean difference: -2.6).

After:

2) Endpoints and Estimates Used to Calculate Target Sample Size

In Phase II/III study (E0302-J081-761), an estimate of the change in ALSFRS-R total score was estimated using data from a subset of patients who experience symptoms for ≤one year at the initiation of the observation period and whose total ALSFRS-R scores decreased by 1-2 points during the observation period (12 weeks). The mean ± SD of the change in ALSFRS-R total score at 16 weeks was-3.2 ± 4.0 in the mecobalamin 50 mg group (26 patients in the E0302 – 50 mg group in this study) and-5.8 ± 5.0 in the placebo group (n = 32) (mean difference: -2.6).

Ver2.0 (2018.3.30)

・**P.9 [Subjects]**

Reason for change: Error

Before:
Eligible patients: Those who meet the following inclusion criteria (1)-(4), (6) and (7) at the initiation of the observation period, and (3) and (5), (7) at the completion of the observation period, and do not meet any of the exclusion criteria (1)-(14) at the initiation of the observation period, and (1)-(4), (7)-(11), (13)-(14) at the completion of the observation period.
After:
Eligible patients: Those who meet the following inclusion criteria (1)-(4), (6) and (7) at the initiation of the observation period, and (3) and (5)-(7) at the completion of the observation period, and do not meet any of the exclusion criteria (1)-(14) at the initiation of the observation period, and (1)-(4), (7)-(11), (13)-(14) at the completion of the observation period.

・**P.9 [Subjects]**
Reason for change: Correction of descriptions
Before:
Exclusion criteria: Patients who meet any one of the following criteria will be excluded.
(6) Patients receiving edaravone within four weeks prior to obtaining informed consent
After:
Exclusion criteria: Patients who meet any one of the following criteria will be excluded.
(6) Patients receiving edaravone within four weeks prior to enrollment in the observation period

・**P.19 4.3. Eligibility Criteria**
Reason for change: Error
Before:
Patients who meet the following inclusion criteria (1)-(4), (6) and (7) at the initiation of the observation period, and (3) and (5), (7) at the completion of the observation period, and do not meet any of the exclusion criteria (1)-(14) at the initiation of the observation period, and (1)-(4), (7)-(11), (13)-(14) at the completion of the observation period are eligible.
After:
Patients who meet the following inclusion criteria (1)-(4), (6) and (7) at the initiation of the observation period, and (3) and (5)-(7) at the completion of the observation period, and do not meet any of the exclusion criteria (1)-(14) at the initiation of the observation period, and (1)-(4), (7)-(11), (13)-(14) at the completion of the observation period are eligible.

・**P.20 4.3.2. Exclusion Criteria**
Reason for change: Correction of descriptions
Before:
Exclusion criteria: Patients who meet any one of the following criteria will be excluded.
(6) Patients receiving edaravone within four weeks prior to obtaining informed consent
After:
Exclusion criteria: Patients who meet any one of the following criteria will be excluded.
(6) Patients receiving edaravone within four weeks prior to enrollment in the observation period

・**P.29 8.2.3. Confirmation of Assignment Status**
Reason for change: Correction of descriptions
Before:
The Medical Statistical Advisor receives reports from the registration center on the status of assignment (subject characteristics by treatment group) masked by the treatment group name as appropriate and, if there is an imbalance in the allocation adjustment factor (minimization factor) and the other important prognostic factors, recommends a change in the method of assignment to the coordinating investigator and the registration center along with the response measures.
After:

The Medical Statistical Advisor receives reports from the registration center on the status of assignment (subject characteristics by treatment group) masked by the treatment group name as appropriate and, if there is an imbalance in the allocation adjustment factor (minimization factor), recommends a change in the method of assignment to the coordinating investigator and the registration center along with the response measures.

・P.34 9.2.1.1. Observation period Week 0 (Initiation of the observation period)

Reason for change: Correction of descriptions

Before:

(1) Patient characteristics at the initiation of the observation period

6) History of the present illness

・ History of administration of edaravone more than four weeks prior to obtaining informed consent in the observation period (including reasons for switching)

After:

(1) Patient characteristics at the initiation of the observation period

6) History of the present illness

・ History of administration of edaravone more than four weeks prior to enrollment in the observation period (including reasons for switching)

・P.63 18.2.3.2. Data Monitoring

Reason for change: Correction of descriptions

Before:

The Medical Statistical Advisor will receive a report from the registration center of the allocation status (distribution of subject characteristics by group) with the name of the group, as appropriate. If there is an imbalance in the allocation adjustment factor (minimization factor) and the other important prognostic factors (Continuous amount of variables categorized as minimization factors, such as the total ALSFRS-R score at the end of the observation period and the change in ALSFRS-R during the observation period), the change in the allocation method will be recommended to the coordinating investigator along with the response measures.

After:

The Medical Statistical Advisor will receive a report from the registration center of the allocation status (distribution of subject characteristics by group) with the name of the group, as appropriate. If there is an imbalance in the allocation adjustment factor (minimization factor), the change in the allocation method will be recommended to the coordinating investigator along with the response measures.

Ver3.0 (2018.7.4)

・P.4 TABLE OF CONTENTS

Reason for change: Correction of descriptions

Before:

6.2.1. Prohibited Concomitant Drugs

After:

6.2.1. Prohibited Concomitant Drugs and Therapy

・P.14 3.2. Study Flow

Reason for change: Correction of descriptions

Before:

In the Continuous treatment period, independent evaluation is not required, and safety and efficacy evaluations, surveys, and administration of the investigational product are permitted by the principal investigator and others.

After:
In the Continuous treatment period, independent evaluation is not required, and all evaluations, surveys, and administration of the investigational product are permitted by the principal investigator and others.

・**P.25 5.6.1.1. During the Continuous Treatment Period**
Reason for change: Add the description about the maximum numbers of investigational products prescribed at once
Before:
In the considerations for storing the investigational products at the subject's home (see 5.6.2.1. During the Treatment Period), it is not required to lock if it is self-administered.
After:
In the considerations for storing the investigational products at the subject's home (see 5.6.2.1. During the Treatment Period), it is not required to lock if it is self-administered. If the investigational products are stored at the subject's home, the maximum number of investigational drugs that can be prescribed is 24.

・**P.26 6.2. Concomitant Therapy**
Reason for change: Correction of descriptions
Before:
6.2.1. Prohibited Concomitant Drugs
After:
6.2.1. Prohibited Concomitant Drugs and Therapy

・**P.26 6.2.1. Prohibited Concomitant Drugs and Therapy**
Reason for change: Correction of descriptions
Before:
The following drugs will be prohibited from the initiation of the observation period to Week 16 of the Treatment period or at the time of discontinuation.
1) Drugs with Possible Efficacy in ALS (Appendix 2)
2) Drugs whose main ingredient is vitamin B12 (excluding topical agents) (Appendix 2)
3) Other investigational products, investigational products such as regenerative medicine, and investigational devices
[Rationale]
1) (2) Since this product may affect the evaluation of the efficacy of the investigational product, it was set.
3) Considerations for the safety of subjects were included.
After:
The following drugs will be prohibited from the initiation of the observation period to Week 16 of the Treatment period or at the time of discontinuation, whichever comes first.
1) Drugs with Possible Efficacy in ALS (Appendix 2)
2) Drugs whose main ingredient is vitamin B12 (excluding topical agents) (Appendix 2)
3) Other investigational products, investigational products such as regenerative medicine, and investigational devices
[Rationale]
1) (2) Since this product may affect the evaluation of the efficacy of the investigational product, it was set.
3) Considerations for the safety of subjects were included.

The following therapies are prohibited from the initiation of the observation period to Week 16 of the Treatment period or at the time of discontinuation, whichever comes first.

4) HAL medical leg type (Appendix 2)

・**P.26 6.2.2. Restricted Concomitant Drug**
Reason for change: Correction of descriptions
Before:
However, the daily dose of riluzole should not be changed or administered at the initiation of the observation period until Week 16 of treatment or at the time of discontinuation.
After:
However, the daily dose of riluzole should not be changed or administered at the initiation of the observation period until Week 16 of treatment or at the time of discontinuation, whichever comes first.

・**P.26 6.2.3. Concomitant Therapy**
Reason for change: Correction of descriptions
Before:
1) Rehabilitation can be performed at the initiation of the observation period until Week 16 of treatment or at the time of discontinuation. Active rehabilitation should be provided according to the subject's condition, including exercise and respiratory training to restore muscle strength (see 10.7 Concomitant Medications and Concomitant Therapies). However, the HAL medical leg type is prohibited from the initiation of the observation period to week16 of the Treatment period or at the time of discontinuation, whichever comes first.
2) Nutritional management
Even if nutritional support measures (e.g., nasogastric tube feeding, IVH, or PEG) are administered at the initiation of the observation period until Week 16 of treatment or at the time of discontinuation, a continuation of the study is allowed (see 10.7 Concomitant Medications/Therapies).
After:
1) Rehabilitation can be performed during all periods of the Observation, Treatment, and Continuous treatment periods. Active rehabilitation should be provided according to the subject's condition, including exercise and respiratory training to restore muscle strength (see 10.7 Concomitant Medications and Concomitant Therapies). However, the HAL medical leg type is prohibited from the initiation of the observation period to week16 of the Treatment period or at the time of discontinuation, whichever comes first.
2) Nutritional management
Even if nutritional support measures (e.g., nasogastric tube feeding, IVH, or PEG) are administered during all periods of the Observation, Treatment, and Continuous treatment periods, a continuation of the study is allowed (see 10.7 Concomitant Medications/Therapies).

・**P.33 9.1. Study Schedule**
Reason for change: Correction of descriptions
Before:

Timing		Observation period		Treatment period (double-blinded)				Contunious treatment period
		Week 0 Initiation	12 weeks (end)	Week 0 (Initial day of administration*1)	4 weeks	8 weeks	16 weeks (Completed/Discontinued)	Every 12 weeks (Completed/Discontinued)
Acceptable range (weeks)		—	±1				—	±2
				—	±1	±1	±2	
Tests, observations, and assessments	Obtainment of written informed consent	●					●*2	
	Patient Characteristics	●	●					
	Registration of the observation period	●						
	Enrollment and assignment during the		●					
	Diagnosis (including electromyography and	●*10	●					
	Event occurrence*3			←	—	—	—	→
	ALSFRS-R	●	●		●	●	●	●
	%FVC	●	●			●	●	
	MMTs; handgrip strength testing; Norris		●*4			●	●	
	Blood homocystei ne (intensive		●				●	
	Clinical Laboratory Tests (Hematology,	●*5	●*6		●*7		●*7	●
	12-lead ECG	●	●*8	●* 8, 9			●*9	●
	Vital signs	●	●		●	●	●	●
	Administration of the investigational drug			With E0302 - 50 mg/dose or placebo Intramuscular injection twice weekly*13				E0302 - 50 mg/dose Intramuscular injection twice weekly*13
	Treatment conditions for investigational			←	—	—	—	→
	Tracheostomy status			←	—	—	—	→
	Concomitant medication/treatment	←	—	—	—	—	—	→
	Adverse events*3	←	—	—	—	—	—	→

*1 Conducted from Day 0 (Allocation date) to Day3. *2 Obtained informed consent from Week 12 to Week 16. *3 Occurrence of the Event and Adverse event are required to be reported from the last administration date to 28 days later. *4 Conducted in the eligible patients for the treatment period. *5 Measurement of serum Vitamin B12 level are conducted. *6 Women only take a pregnancy test. *7 Conducted before administration of the investigational product. *8 QT assessments are conducted before administration of the investigational product if the first administration is on the allocation date. *9 QT assessments are conducted twice (before administration and 2 hours later after administration). *10 The results of electromyography and nerve conduction studies conducted in the other medical institutions allowed to be evaluated.

After:

Timing		Observation period		Treatment period (double-blinded)				Contunious treatment period
		Week 0 Initiation	12 weeks (end)	Week 0 (Initial day of administration*1)	4 weeks	8 weeks	16 weeks (Completed/Discontinued)	Every 12 weeks (Completed/Discontinued)
Acceptable range (weeks)		—	±1				—	±2
				—	±1	±1	±2	
Tests, observations, and assessments	Obtainment of written informed consent	●					●*2	
	Patient Characteristics	●	●					
	Registration of the observation period	●						
	Enrollment and assignment during the		●					
	Diagnosis (including electromyography and	●*11	●					
	Event occurrence*3			←	—	—	—	→
	ALSFRS-R	●	●		●	●	●	●
	%FVC	●	●			●	●	
	MMTs; handgrip strength testing; Norris		●*4			●	●	
	Blood homocystei ne (intensive		●				●	
	Clinical Laboratory Tests (Hematology,	●*5	●*6		●*7		●*7	●
	12-lead ECG	●	●*8	●* 8, 9			●*9, *10	●
	Vital signs	●	●		●	●	●	●
	Administration of the investigational drug			With E0302 - 50 mg/dose or placebo Intramuscular injection twice weekly*13			E0302 - 50 mg/dose Intramuscular injection twice weekly*13	
	Treatment conditions for investigational			←	—	—	—	→
	Tracheostomy status			←	—	—	—	→
	Concomitant medication/treatment	←	—	—	—	—	—	→
	Adverse events*3	←	—	—	—	—	—	→

*1 Should be conducted from Day 0 (Allocation date) to Day3. *2 Obtained informed consent from Week 12 to Week 16. *3 Occurrence of the Event and Adverse event are required to be reported from the last administration date to 28 days later. *4 Conducted in the eligible patients for the treatment period. *5 Measurement of serum Vitamin B12 level are conducted. *6 Women only take a pregnancy test. *7 Conducted before administration of the investigational product. *8 QT assessments are conducted before administration of the investigational product if the first administration is on the allocation date. *9 QT assessments are conducted twice (before administration and 2 hours later after administration). *10 Conducted from Week 8 to the last administration date of Week 15. *11 The results of electromyography and nerve conduction studies conducted in the other medical institutions allowed to be evaluated.

・**P.34 9.2.1.1. Observation period Week 0 (Initiation of the observation period)**

Reason for change: Correction of descriptions

Before:

(1) Patient characteristics at the initiation of the observation period

7) Complications

Diseased under treatment at the initiation of the observation period

After:

(1) Patient characteristics at the initiation of the observation period

7) Complications

Diseased at the initiation of the observation period

・**P.35 9.2.1.2. Observation period Week 12 (Completion of the observation period)**

Reason for change: Correction of descriptions

Before:

(2) Efficacy endpoint

7) Concentration of homocysteine in the blood (conducted in the eligible patients for the treatment period)
After:
(2) Efficacy endpoint
7) Concentration of homocysteine in the blood

・**P.36 9.2.2.2. Week 4, 8, 16, and Discontinuation of Study**
Reason for change: Correction of descriptions
Before:
(2) Safety endpoint
2) 12-Lead ECG (performed once between Week 8 and Week 16 of treatment or at the time of discontinuation)
After:
(2) Safety endpoint
2) 12-Lead ECG (performed once between Week 8 of treatment and the last day of treatment at Week 15 or at the time of discontinuation)

・**P.37 10. Evaluation**
Reason for change: Correction of descriptions
Before:
On the basis of the statement in this section, efficacy, ALSFRS, and safety evaluators independently conduct evaluations and surveys until Week 16 of the Treatment period. Independent evaluation is not required during the Continuous treatment period, and all evaluations and investigations of the investigational product are permitted by the investigator and others.
After:
On the basis of the statement in this section, efficacy, ALSFRS, and safety evaluators independently conduct evaluations and surveys until Week 16 of the Treatment period. Independent evaluation is not required during the Continuous treatment period, and all evaluations, investigations, and administration of the investigational product are permitted by the investigator and others.

・**P.42 10.3.2. Electrocardiogram (ECG)**
Reason for change: Correction of descriptions
Before:
During the treatment period, ECG measurements will be performed twice at 1-minute intervals prior to and 2 hours (±1 hour) after dosing, during the first infusion, and between Week 8 (±1 week) and Week 16 (or discontinuation). As far as possible, this ECG measurement should be performed at the same time period, and the patient will be fasted for at least 8 hours prior to the administration on the day of the visit. In addition, the ECG results during the Treatment period will be sent to the Study Coordinating Office. The ECG should be interpreted by a blinded cardiologist. The results of the reading will be forwarded to the investigator etc., as a report. Corrected intervals to be reported by the cardiologist to the principal investigator, etc., shall be QTcB and QTcF.
After:
During the treatment period, ECG measurements will be performed twice at 1-minute intervals prior to and 2 hours (±1 hour) after dosing, during the first infusion, and between Week 8 (±1 week) and Week 15, the last injection (or discontinuation). As far as possible, this ECG measurement should be performed at the same time period, and the patient will be fasted for at least 8 hours prior to the administration on the day of the visit. In addition, the ECG results during the Treatment period will be sent to the Study Coordinating Office. The ECG should be interpreted by a blinded cardiologist. The results of the reading will be forwarded to the investigator etc., as a report. Corrected intervals to be reported by the cardiologist to the principal investigator, etc., shall be QTcB and QTcF.
Electrocardiogram measurements at the time of discontinuation during the Treatment period are to

be performed once, regardless of whether a meal is used. There is also no need to report to a cardiologist.

・**P.48 12.4.1. Reporting of Serious Adverse Events**
Reason for change: Correction of descriptions
Before:
1) The investigator or sub-investigator should provide the head of each medical institution and the coordinating investigator with the primary report (within 24 hours of knowledge: within one working day at the latest), secondary report (within seven days), detailed investigation report, and final report.
After:
1) The investigator or sub-investigator should provide the head of each medical institution and the coordinating investigator with the primary report (within 24 hours of knowledge: within one working day at the latest), secondary report (within seven days: not mandatory if a full report is included in the primary report), detailed investigation report, and final report.

・**P.53 13.1. Criteria for Discontinuation of Study**
Reason for change: Correction of descriptions
Before:
10) Use of prohibited concomitant drugs or therapies during the period from the initiation of the observation period to Week 16 of the Treatment period (refer to "6.2.1 Prohibited Concomitant Drugs").
After:
10) Use of prohibited concomitant drugs or therapies during the period from the initiation of the observation period to Week 16 of the Treatment period (refer to "6.2.1 Prohibited Concomitant Drugs/Treatments" and "6.2.3 Concomitant Therapies").

・**P.66 Study Cost**
Reason for change: Correction of descriptions
Before:
Since this clinical trial applies the non-insurance combined medical care expense system, the health insurance of the examinee is adapted under this system except for the cost related to the administration of the investigational drug. The investigational product will be provided free of charge by the supplier of the investigational product, and the expenses related to the administration of the investigational product will be paid through research funds. In addition, measurements of the concentration of homocysteine in the blood and pregnancy tests are paid through research funds.
After:
Since this clinical trial applies the non-insurance combined medical care expense system, the health insurance of the examinee is adapted under this system except for the cost related to the administration of the investigational drug. The investigational product will be provided free of charge by the supplier of the investigational product, and the expenses related to the administration of the investigational product will be paid through research funds. In addition, measurements of the concentration of homocysteine in the blood and pregnancy tests are paid through research funds.
When paying the clinical trial cooperative expenses, expenses for reducing the burden, etc. to the subjects, these shall be paid in accordance with the provisions of each participating medical organization

Ver4.0 (2018.11.6)

2701

・**P.17 4.1. Diagnostic criteria**

Reason for change: Correction of descriptions

Before:

The El Escorial revised Airlie House diagnostic criteria are shown below.[16, 17] Diagnostic flow is shown in Figure 4-1.

-

(3) Clinically probable and laboratory evidence of ALS (clinically probable-laboratory-supported ALS) is defined as clinical signs of upper and lower motor neuron dysfunction in one region together with neurophysiological evidence of lower motor neuron dysfunction in 2 regions.

After:

The El Escorial revised Airlie House diagnostic criteria are shown below.[16, 17]

-

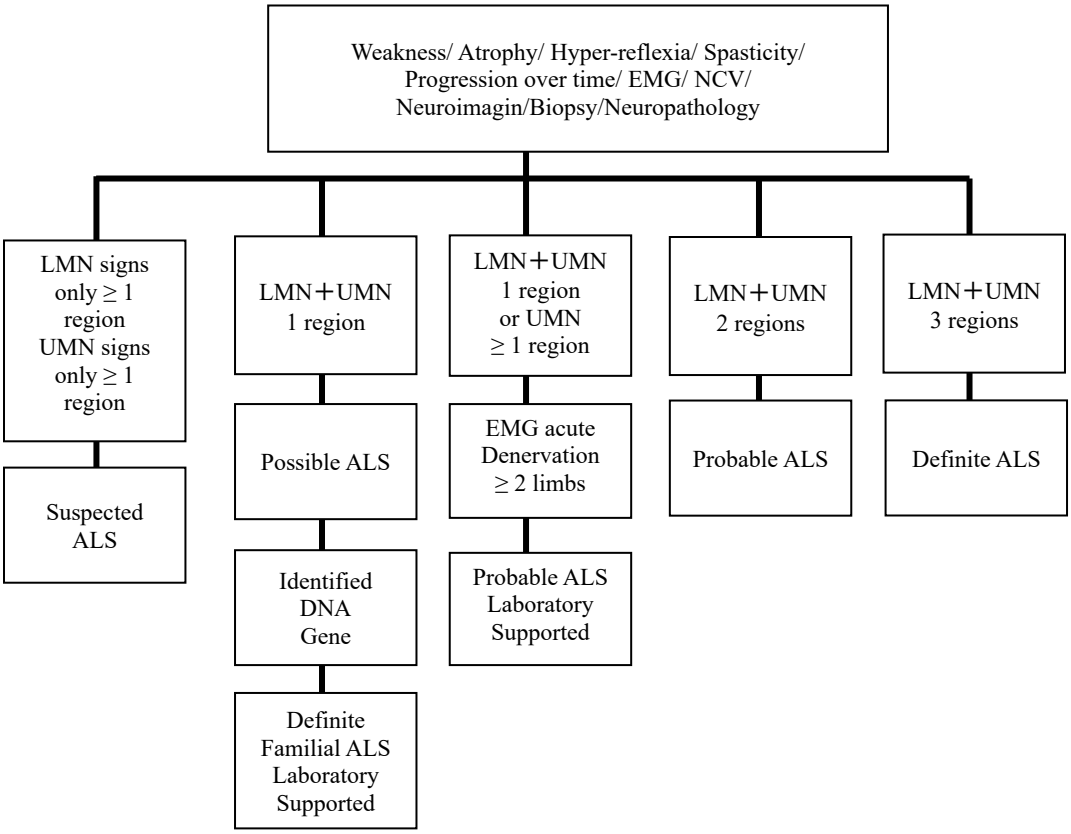
(3) Clinically probable and laboratory evidence of ALS (clinically probable-laboratory-supported ALS) is defined as clinical signs of upper and lower motor neuron dysfunction in one region together with neurophysiological evidence of lower motor neuron dysfunction (Positive sharp wave, Fibrillation potential) in 2 regions.

・**P.18 4.1. ALS diagnostic flow**

Reason for change: Correction of descriptions

Before:

Figure 4-1. ALS diagnostic flow (World Federation of Neurology revised El Escorial criteria 1998)



After: Figure I deleted.

・**P.23 5.5.2. Method of Preparation and Administration**

Reason for change: Correction of descriptions
Before:
The investigational drug administrator will administer the investigational drug twice weekly on an outpatient basis in accordance with the instructions for administration provided by the safety assessor. If the subject becomes unable to visit the hospital as an outpatient, the investigational product may be administered by a coordinator, such as a family physician, who has been designated as an investigational product administrator at the subject's home or nearby medical institution.
After:
The investigational drug administrator will administer the investigational drug twice weekly on an outpatient basis in accordance with the instructions for administration provided by the safety assessor. If the subject becomes unable to visit the hospital as an outpatient, the investigational product may be administered by a coordinator, such as a family physician, who has been designated as an investigational product administrator at the subject's home or nearby medical institution. The investigational product will be administered twice weekly during the 7-day period, starting from the initial day of administration in the Treatment period and Continuous treatment period. It is not possible to administer two doses (four vials) during the same day with at least one dosing interval.

・**P.23 7.2. Obtaining Written Informed Consent**
Reason for change: Correction of descriptions
Before:
4） Obtaining consent for the transition to the Continuous treatment period
If the subject wishes to continue treatment, written informed consent will be obtained from the subject or from the witness at Week 16 of the treatment period.
After:
4） Obtaining consent for the transition to the Continuous treatment period
If the subject wishes to continue treatment, written informed consent will be obtained from the subject or from the witness between Week 12 of treatment and the initiation of the Continuous treatment period.

・**P.23 8.2.1. Procedures for Subject Registration and Investigational drug Assignment**
Reason for change: Correction of descriptions
Before:
-
After:
5) The investigator or sub-investigator will promptly enter the required information in the case registration system on the website after confirming the discontinuation and completion of treatment for withdrawals, treatment discontinuations, and patients who have completed the Treatment period respectively.

・ **P.32 9. 1. Study Schedule**

Reason for change: Correction of descriptions

Before:

Timing		Observation period		Treatment period (double-blinded)				Contunious treatment period
		Week 0 Initiation	12 weeks (end)	Week 0 (Initial day of administration*1)	4 weeks	8 weeks	16 weeks (Completed/Discontinued)	Every 12 weeks (Completed/Discontinued)
Acceptable range (weeks)		—	± 1	—	± 1	± 1	—	± 2
				—	± 1	± 1	± 2	
Tests, observations and assessments	Obtainment of written informed consent	●					●*2	
	Patient Characteristics	●	●					
	Registration of the observation period	●						
	Enrollment and assignment during the Treatment period		●					
	Diagnosis (including electromyography and nerve conduction studies)	●*11	●					
	Event occurrence*3							
	ALSFRS-R	●	●		●	●	●	●
	%FVC	●	●			●	●	
	MMTs; handgrip strength testing; Norris scale; ALSAQ-40		●*4			●	●	
	Blood homocysteine (intensive measurement)		●				●	
	Clinical Laboratory Tests (Hematology, Biochemistry, Urine)	●*5	●*6		●*7		●*7	●
	12-lead ECG	●	●*8	●* 8, 9			●*9, *10	●
	Vital signs	●	●		●	●	●	●
	Administration of the investigational drug			With E0302 - 50 mg/dose or placebo Intramuscular injection twice weekly				E0302 - 50 mg/dose Intramuscular injection twice weekly
	Treatment conditions for investigational drug							
	Tracheostomy status							
	Concomitant medication/treatment							
	Adverse events*3							

*1 Should be conducted from Day 0 (Allocation date) to Day3. *2 Obtained informed consent from Week 12 to Week 16. *3 Occurrence of the Event and Adcerse event are required to be reported from the last administration date to 28 days later. *4 Conducted in the eligible patients for the treatment period. *5 Mesurement of serum Vitamin B12 level are conducted. *6 Women only take a pregnancy test. *7 Conducted before administration of the investigational product. *8 QT assessment are conducted before administration of the investigational product, if the first administration is on the allocation date. *9 QT assessment are conducted twice (before administration and 2 hours later after administration). *10 Conducted from Week 8 to the last administration date of Week 15. *11 The results of electromyography and nerve conduction studies conducted in the other medical institutions allowed to be evaluated.

After:

Timing		Observation period		Treatment period (double-blinded)				Contunious treatment period
		Week 0 Initiation	12 weeks (end)	Week 0 (Initial day of administration*1)	4 weeks	8 weeks	16 weeks (Completed/Discontinued)	Every 12 weeks (Completed/Discontinued)
Acceptable range (weeks)		—	± 1				—	± 2
				—	± 1	± 1	± 2	
Tests, observations and assessments	Obtainment of written informed consent	●					●*2	
	Patient Characteristics	●	●					
	Registration of the observation period	●						
	Enrollment and assignment during the Treatment period		●					
	Diagnosis (including electromyography and nerve conduction studies)	●*11	●					
	Event occurrence*3							
	ALSFRS-R*12	●	●		●	●	●	●
	%FVC	●	●			●	●	
	MMTs; handgrip strength testing; Norris scale; ALSAQ-40		●*4			●	●	
	Blood homocysteine (intensive measurement)		●				●	
	Clinical Laboratory Tests (Hematology, Biochemistry, Urine)	●*5	●*6		●*7		●*7	●
	12-lead ECG	●	●*8	●* 8, 9			●*9, *10	●
	Vital signs	●	●		●	●	●	●
	Administration of the investigational drug			With E0302 - 50 mg/dose or placebo Intramuscular injection twice weekly*13				E0302 - 50 mg/dose Intramuscular injection twice weekly*13
	Treatment conditions for investigational drug							
	Tracheostomy status							
	Concomitant medication/treatment *12							
	Adverse events*3							

*1 Should be conducted from Day 0 (Allocation date) to Day3. *2 Obtained informed consent from Week 12 to Week 16. *3 Occurrence of the Event and Adcerse event are required to be reported from the last administration date to 28 days later. *4 Conducted in the eligible patients for the treatment period. *5 Mesurement of serum Vitamin B12 level are conducted. *6 Women only take a pregnancy test. *7 Conducted before administration of the investigational product. *8 QT assessment are conducted before administration of the investigational product, if the first administration is on the allocation date. *9 QT assessment are conducted twice (before administration and 2 hours later after administration). *10 Conducted from Week 8 to the last administration date of Week 15. *11 The results of electromyography and nerve conduction studies conducted in the other medical institutions allowed to be evaluated. *12 Among the patients enrolled in the treatment phase, ALSFRS-R evaluation and investigation of concomitant medication and concomitant therapy will be conducted as much as possible until Week 16 of the treatment phase for the discontinued patients except for the untreated patients (except for those who refuse to continue participation in the study or withdraw consent). *13 For twice-weekly administration of the study drug, the drug will be administered twice during a 7-day period starting from the start date of the treatment/continuation period. The interval between doses should be at least one day, and two doses (4 vials) on the same day is not allowed.

・P.35 9.2.1.2. Observation period Week 12 (Completion of the observation period)

Reason for change: Correction of descriptions

Before:

(2) Efficacy endpoint

1 ）ALSFRS-R

2 ）%FVC

3 ）MMT

4 ）Grip strength test

5） Norris scale

6） ALSAQ 40

7） Concentration of homocysteine in the blood

After:

(2) Efficacy endpoint

1） ALSFRS-R

2） %FVC

3） MMT (performed only eligible for treatment)

4） Grip strength test (performed only in eligible patients for treatment)

5） Norris scale (performed only for eligible patients in the treatment period)

6） ALSAQ 40 (performed only in eligible patients for treatment)

7） Concentration of homocysteine in the blood

・P.53 13.2. Procedures for Discontinuation of Study

Reason for change: Correction of descriptions

Before:

-

After:

Among patients enrolled in the treatment period, ALSFRS-R evaluations and investigations of concomitant drugs/therapies will be performed as much as possible until Week 16 of the Treatment period unless patients discontinued from the Treatment period, excluding patients who have not received treatment unless they refuse to continue participation in the study or have requested to withdraw consent. If ambulatory is difficult, the ALSFRS-R will be assessed by a telephone survey, and concomitant medications/therapies will be surveyed in reference to the ALSFRS-R telephone survey flow diagram.

・P.60 18.2.1.6. Handling of Data

Reason for change: Correction of descriptions

Before:

7) Handling of Discontinuation Cases

Discontinuations will be censored on the day of discontinuation. In addition, if an event occurs within 28 days of the last day of administration of the investigational product (the last day of administration of the investigational product is day 0) in a patient who is withdrawn from the study, the efficacy data measured after the event will be analyzed.

After:

7) Handling of Discontinuation Cases

Discontinuations will be censored on the day of discontinuation. However, ALSFRS-R evaluations and investigations of concomitant drugs/therapies will be performed as much as possible until Week 16 of the Treatment period for the patients enrolled in the Treatment period, but not those who discontinued from the Treatment period unless they refuse to continue participation in the study or have requested to withdraw consent. In addition, if an event occurs within 28 days of the last day of administration of

the investigational product (the last day of administration of the investigational product is day 0) in a patient who is withdrawn from the study, the efficacy data measured after the event will be analyzed.

Ver4.1 (2019.2.6)

・**P.53 13.2. Procedures for Discontinuation of Study**

Reason for change: Correction of descriptions

Before:

The investigator or sub-investigator should promptly inform the subject of the termination of the study and provide appropriate medical care and take other necessary measures. The predetermined parameters at the time of discontinuation will be investigated and evaluated within two weeks of the day of administration immediately prior to discontinuation (within four weeks after the day of discontinuation if discontinuation occurs due to withdrawal of at least 15 days). In addition, the date of discontinuation (the day on which the physician judged the discontinuation) and the reason thereof are recorded in the Case Report Form.

After:

The investigator or sub-investigator should promptly inform the subject of the termination of the study and provide appropriate medical care and take other necessary measures. The predetermined parameters at the time of discontinuation (the day on which the physician judged the discontinuation) and the reason thereof is recorded in the Case Report Form. The predetermined parameters will be investigated and evaluated after the last dose of the investigational drug and within ±2 weeks from the date of discontinuation.

Ver5.0 (2019.4.24)

・**P.53 13.2. Procedures for Discontinuation of Study**

Reason for change: Correction of descriptions

Before:

In this study, when ambulatory visits are difficult due to the patient's reasons, such as the reason for progression of the underlying disease during the Continuous treatment period, because of the patient's health status, etc., administration of the investigational product at the subject's home (self-administration) may be performed by subjects or their families trained in the administration of the investigational product in addition to the administration at the subject's home or nearby medical institutions. In the considerations for storing the investigational products at the subject's house (see "5.6.2.1. During the Treatment Period"), it is not required to lock if it is self-administered. If the investigational products are stored at the subject's home, the maximum number of investigational drugs that can be prescribed is 24.

After:

In this study, when ambulatory visits are difficult due to the patient's reasons, such as the reason for progression of the underlying disease during the Continuous treatment period, because of the patient's health status, etc., administration of the investigational product at the subject's home (self-administration) may be performed by subjects or their families trained in the administration of the investigational product in addition to the administration at the subject's home or nearby medical institutions. In the considerations for storing the investigational products at the subject's house (see "5.6.2.1. During the Treatment Period"), it is not required to lock if it is self-administered. If the investigational products are stored at the subject's home, the maximum number of investigational drugs that can be prescribed is 28.

・**P.53 9.1. Study schedule**

Reason for change: Correction of descriptions

Before:

Table 9-1 Study Schedule

Timing		Observation period		Treatment period (double-blinded)				Continuous treatment period
		Week 0 Initiation	12 weeks (end)	Week 0 (Initial day of administration*1)	4 weeks	8 weeks	16 weeks (Completed/Discontinued)	Every 12 weeks (Completed/Discontinued)
Acceptable range (weeks)		—	± 1	—	± 1	± 1	—	± 2
							± 2	
Tests, observations, and assessments	Obtainment of written informed consent	●					●*2	
	Patient Characteristics	●	●					
	Registration of the observation period	●						
	Enrollment and assignment during the Treatment period		●					
	Diagnosis (including electromyography and nerve conduction studies)	●*11	●					
	Event occurrence*3							
	ALSFRS-R*12	●	●		●	●	●	●
	%FVC	●	●			●	●	
	MMTs; handgrip strength testing; Norris scale; ALSAQ-40		●*4			●	●	
	Blood homocysteine (intensive measurement)		●				●	
	Clinical Laboratory Tests (Hematology, Biochemistry, Urine)	●*5	●*6		●*7		●*7	●
	12-lead ECG	●	●*8	●* 8, 9			●*9, *10	●
	Vital signs	●	●		●	●	●	●
	Administration of the investigational drug			With E0302 - 50 mg/dose or placebo Intramuscular injection twice weekly*13				E0302 - 50 mg/dose Intramuscular injection twice weekly*13
	Treatment conditions for investigational drug							
	Tracheostomy status							
	Concomitant medication/treatment *12							
	Adverse events*3							

*1 Should be conducted from Day 0 (Allocation date) to Day3. *2 Obtained informed consent from Week 12 to Week 16. *3 Occurrence of the Event and Adcerse event are required to be reported from the last administration date to 28 days later. *4 Conducted in the eligible patients for the treatment period. *5 Mesurement of serum Vitamin B12 level are conducted. *6 Women only take a pregnancy test. *7 Conducted before administration of the investigational product. *8 QT assessment are conducted before administration of the investigational product, if the first administration is on the allocation date. *9 QT assessment are conducted twice (before administration and 2 hours later after administration). *10 Conducted from Week 8 to the last administration date of Week 15. *11 The results of electromyography and nerve conduction studies conducted in the other medical institutions allowed to be evaluated. *12 Among the patients enrolled in the treatment phase, ALSFRS-R evaluation and investigation of concomitant medication and concomitant therapy will be conducted as much as possible until Week 16 of the treatment phase for the discontinued patients except for the untreated patients (except for those who refuse to continue participation in the study or withdraw consent). *13 For twice-weekly administration of the study drug, the drug will be administered twice during a 7-day period starting from the start date of the treatment/continuation period. The interval between doses should be at least one day, and two doses (4 vials) on the same day is not allowed.

After:

Table 9-1 Study Schedule

Timing		Observation period		Treatment period (double-blinded)				Continuous treatment period	Discontinuation (Treatment period/ Continuous treatment period)
		Week 0 Initiation	12 weeks (end)	Week 0 (Initial day of administration*1)	4 weeks	8 weeks	16 weeks (Completed/Discontinued)	Every 12 weeks (Completed/Discontinued)	
Acceptable range (weeks)		—	±1	—	±1	±1	—	±2	±2*14
							±2		
Tests, observations, and assessments	Obtainment of written informed consent	●					●*2		
	Patient Characteristics	●	●						
	Registration of the observation period	●							
	Enrollment and assignment during the Treatment period		●						
	Diagnosis (including electromyography and nerve conduction studies)	●*11	●						
	Event occurrence*3								
	ALSFRS-R*12	●	●		●	●	●	●	●
	%FVC	●	●			●	●		
	MMTs; handgrip strength testing; Norris scale; ALSAQ-40		●*4			●	●		
	Blood homocysteine (intensive measurement)		●				●		
	Clinical Laboratory Tests (Hematology, Biochemistry, Urine)	●*5	●*6		●*7		●*7	●	●
	12-lead ECG	●	●*8	●* 8, 9			●*9, *10	●	
	Vital signs	●	●		●	●	●	●	●
	Administration of the investigational drug			With E0302 - 50 mg/dose or placebo Intramuscular injection twice weekly*13				E0302 - 50 mg/dose Intramuscular injection twice weekly*13	
	Treatment conditions for investigational drug								
	Tracheostomy status								
	Concomitant medication/treatment *12								
	Adverse events*3								

*1 Should be conducted from Day 0 (Allocation date) to Day3. *2 Obtained informed consent from Week 12 to Week 16. *3 Occurrence of the Event and Adcerse event are required to be reported from the last administration date to 28 days later. *4 Conducted in the eligible patients for the treatment period. *5 Mesurement of serum Vitamin B12 level are conducted. *6 Women only take a pregnancy test. *7 Conducted before administration of the investigational product. *8 QT assessment are conducted before administration of the investigational product, if the first administration is on the allocation date. *9 QT assessment are conducted twice (before administration and 2 hours later after administration). *10 Conducted from Week 8 to the last administration date of Week 15. *11 The results of electromyography and nerve conduction studies conducted in the other medical institutions allowed to be evaluated. *12 Among the patients enrolled in the treatment phase, ALSFRS-R evaluation and investigation of concomitant medication and concomitant therapy will be conducted as much as possible until Week 16 of the treatment phase for the discontinued patients except for the untreated patients (except for those who refuse to continue participation in the study or withdraw consent). *13 For twice-weekly administration of the study drug, the drug will be administered twice during a 7-day period starting from the start date of the treatment/continuation period. The interval between doses should be at least one day, and two doses (4 vials) on the same day is not allowed. ※14 Performed after last administration of the investigational drug and within ± 2 weeks from the discontinuation date.

Ver6.0 (2019.12.27)

・**P.9 Sample Size and Duration**

Reason for change: Extension of the continuous treatment period

Before:

The target number of subjects: 128 (64 in the placebo group and 64 in the E0302 - 50 mg group)

Planned study period: October 2017 to March 2020 (planned)

Planned period for case registration: October 2017 to September 2019 (scheduled)

Period for each subject: From the date of obtainment of informed consent to Week 16 of the Treatment period (subjects wishing to continue treatment beyond Week 16 move to the Continuous treatment period (until March 2020) after obtaining informed consent.

After:

The target number of subjects: 128 (64 in the placebo group and 64 in the E0302 - 50 mg group)

Planned study period: October 2017 to March 2022 (planned)

(If no significant difference is found in the primary endpoint, the study will be terminated at that time.)

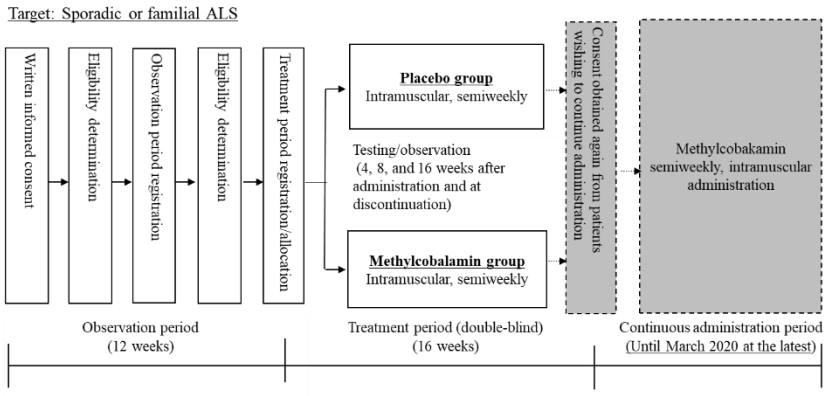
Planned period for case registration: October 2017 to October 2019 (scheduled)

Period for each subject: From the date of obtainment of informed consent to Week 16 of the Treatment period (subjects wishing to continue treatment beyond Week 16 move to the Continuous treatment period (until March 2022) after obtaining informed consent.

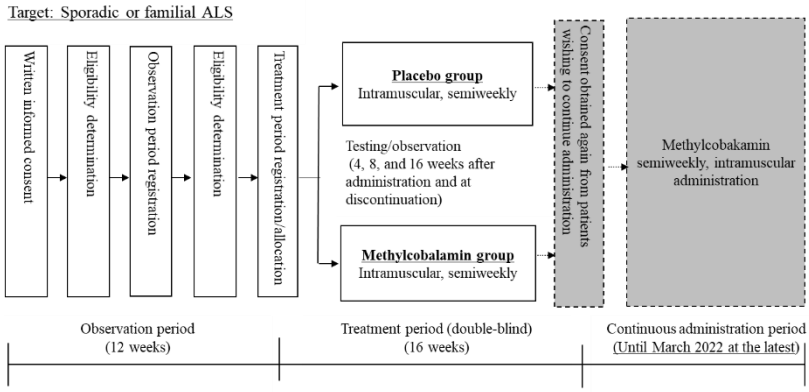
・**P.10 Design of clinical trials**

Reason for change: Extension of the continuous treatment period

Before:



After:



・P.10 Dosage and method of administration

Reason for change: Extension of the continuous treatment period

Before:

E0302 - 50 mg or placebo will be administered intramuscularly twice weekly from the initial day of administration until the completion of the 16-week treatment period.

Subjects who wish to continue treatment beyond Week 16 of the Treatment period will enter the Continuous treatment period and receive an intramuscular dose of E0302 - 50 mg until March 2020.

After:

E0302 - 50 mg or placebo will be administered intramuscularly twice weekly from the initial day of administration until the completion of the 16-week treatment period.

Subjects who wish to continue treatment beyond Week 16 of the Treatment period will enter the Continuous treatment period and receive an intramuscular dose of E0302 - 50 mg until March 2022.

・P.13 2. HISTORY OF DEVELOPMENT (BACKGROUND INFORMATION)

Reason for change: Extension of the continuous treatment period

Before:

On the basis of the results of Phase II/III study conducted by Eisai Co., Ltd., we planned to perform a multicenter, randomized, placebo-controlled, double-blinded, parallel-group study with the primary endpoint of change in the ALSFRS-R total scores from the date of allocation to Week 16. The study will include patients with ALS who had developed ALS within one year after the onset of symptoms at the initiation of the observation period and had a 1-2-point decrease in the total ALSFRS-R score during the observation period (12 weeks). In addition, subjects who wish to continue treatment beyond Week 16 of the Treatment period were allowed to continue treatment with E0302 - 50 mg by moving to the Continuous treatment period until March 2020.

After:

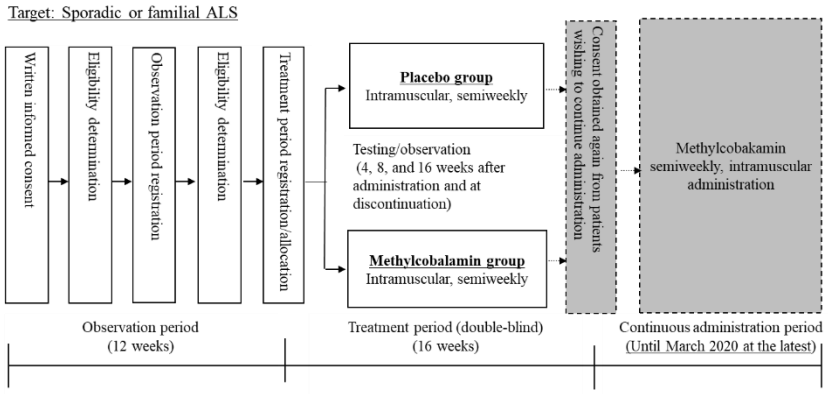
On the basis of the results of Phase II/III study conducted by Eisai Co., Ltd., we planned to perform a multicenter, randomized, placebo-controlled, double-blinded, parallel-group study with the primary endpoint of change in the ALSFRS-R total score from the date of allocation to Week 16. The study will include patients with ALS who had developed ALS within one year after the onset of symptoms at the initiation of the observation period and had a 1-2-point decrease in the total ALSFRS-R score during the observation period (12 weeks). In addition, subjects who wish to continue treatment beyond Week 16 of the Treatment period were allowed to continue treatment with E0302 - 50 mg by moving to the Continuous treatment period until March 2022.

・P.14 3.2.Study Flow

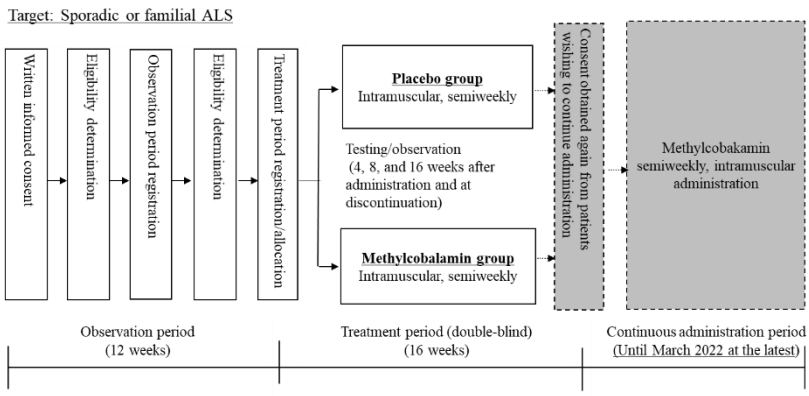
Reason for change: Extension of the continuous treatment period

Before:

Figure 3-1 Schematic of Study Conduct



After:
Figure 3-1 Schematic of Study Conduct



・P.15 3.4.Sample Size and Study Period

Reason for change: Extension of the continuous treatment period
Before:
The target number of subjects: 128 (64 in the placebo group and 64 in the E0302 - 50 mg group)
Study period: October 2017 to March 2020 (planned)
Case registration period: October 2017 to September 2019 (scheduled)
Period for each subject: From the date of informed consent obtainment to Week 16 of the Treatment period (subjects wishing to continue treatment beyond Week 16 move to the Continuous treatment period (until March 2020) after obtaining informed consent.
After:
The target number of subjects: 128 (64 in the placebo group and 64 in the E0302 - 50 mg group)
Study period: October 2017 to March 2022 (planned)
(If no significant difference is found in the primary endpoint, the study will be terminated at that time.)
Case registration period: October 2017 to October 2019 (planned)
Period for each subject: From the date of informed consent obtainment to Week 16 of the Treatment period (subjects wishing to continue treatment beyond Week 16 move to the Continuous treatment period (until March 2022) after obtaining informed consent.

・P.22 5.5.1. Dose and Administration Method

Reason for change: Extension of the continuous treatment period
Before:
E0302 - 50 mg or placebo will be administered intramuscularly twice weekly from the initial day of administration until Week 16 of the Treatment period.
For subjects who wish to enter the Continuous treatment period after the completion of the Treatment period, E0302 - 50 mg will be administered intramuscularly twice weekly from the initial day of the Continuous treatment period until March 2020.
After:
E0302 - 50 mg or placebo will be administered intramuscularly twice weekly from the initial day of administration until Week 16 of the Treatment period.
For subjects who wish to enter the Continuous treatment period after the completion of the Treatment period, E0302 - 50 mg will be administered intramuscularly twice weekly from the initial day of the Continuous treatment period until March 2022.

・**P.22 5.5.3. Treatment Period**

Reason for change: Extension of the continuous treatment period

Before:

The Treatment period is 16 weeks (4 months). In addition, if the subject wishes, the Extension may be continued until March 2020 as the Continuous treatment period.

Even during the Continuous treatment period, treatment will be discontinued at the time of the event.

[Rationale for the setting of dosing period]

In the analysis of the subgroup of Phase II/III study, the difference in the change in the ALSFRS-R total score at four weeks (95% confidence interval) was 0.4 (-0.7, 1.5), which was not significantly different (P = 0.258), but the difference in the change in the ALSFRS-R total score at 16 weeks (95% confidence interval) was 3.3 (0.5, 6.0), which was significant (P = 0.017).

On the other hand, a decrease in the ALSFRS-R total score at 100 days of observation has been reported to be helpful in predicting survival.20 ALSFRS-R assessment at 16 weeks may be predictive of clinically significant survival.

Therefore, it was deemed appropriate to have a 16-week evaluation period, and a shorter 4-week evaluation period would be difficult. Since this study is a placebo-controlled study in subjects with ALS, the Treatment period was set at 16 weeks in order to shorten the placebo administration period as much as possible due to ethical considerations. The extended administration was allowed until March 2020 (Continuous treatment period).

After:

The Treatment period is 16 weeks (4 months). In addition, if the subject wishes, the Extension may be continued until March 2022 as the Continuous treatment period.

Even during the Continuous treatment period, treatment will be discontinued at the time of the event.

[Rationale for the setting of dosing period]

In the analysis of the subgroup of Phase II/III study, the difference in the change in the ALSFRS-R total score at four weeks (95% confidence interval) was 0.4 (-0.7, 1.5), which was not significantly different (P = 0.258), but the difference in the change in the ALSFRS-R total score at 16 weeks (95% confidence interval) was 3.3 (0.5, 6.0), which was significant (P = 0.017).

On the other hand, a decrease in the ALSFRS-R total score at 100 days of observation has been reported to be helpful in predicting survival.20 ALSFRS-R assessment at 16 weeks may be predictive of clinically significant survival.

Therefore, it was deemed appropriate to have a 16-week evaluation period, and a shorter 4-week evaluation period would be difficult. Since this study is a placebo-controlled study in subjects with ALS, the Treatment period was set at 16 weeks in order to shorten the placebo administration period as much as possible due to ethical considerations. The extended administration was allowed until March 2022 (Continuous treatment period).

・**P.22 22.1. Disclosure and Confidentiality**

Reason for change: Add the new funding source

Before:

This clinical trial is adopted in "Research Project for Practical Application of Intractable Diseases" of the National Research and Development Corporation of Japan (AMED) and performed in part using public research funds. Investigational products will be provided free of charge by the supplier of the investigational product. The supplier will not influence the results of this study. The investigator or sub-investigator will not distort his/her professional judgment in the conduct or reporting of the study for financial or other personal benefits. Clinical trials are conducted fairly, and the interests of the manufacturers of products and drugs used in clinical trials are appropriately managed in accordance with the Conflict of Interest Rules of the participating medical organizations.

After:

This clinical trial is adopted in "Research Project for Practical Application of Intractable Diseases" of the National Research and Development Corporation of Japan (AMED) and performed in part

using public research funds. Investigational products will be provided free of charge by the supplier of the investigational product. The supplier will not influence the results of this study. This trial will also be funded by Eisai Co., Ltd. from April 2020 onwards. The investigator or sub-investigator will not distort his/her professional judgment in the conduct or reporting of the study for financial or other personal benefits. Clinical trials are conducted fairly, and the interests of the manufacturers of products and drugs used in clinical trials are appropriately managed in accordance with the Conflict of Interest Rules of the participating medical organizations.

Ver7.0 (2020.6.26)

・P.25 5.6.2.2. During the Continuous Treatment Period

Reason for change: Add the procedures how to deal with COVID-19

Before:

In this study, when ambulatory visits are difficult due to the patient's reasons, such as the reason for progression of the underlying disease during the Continuous treatment period, because of the patient's health status, etc., administration of the investigational product at the subject's home (self-administration) may be performed by subjects or their families trained in the administration of the investigational product in addition to the administration at the subject's home or nearby medical institutions. In the considerations for storing the investigational products at the subject's house (see "5.6.2.1. During the Treatment Period"), it is not required to lock if it is self-administered. If the investigational products are stored at the subject's home, the maximum number of investigational drugs that can be prescribed is 28.

After:

In this study, when ambulatory visits are difficult due to the patient's reasons, such as the reason for progression of the underlying disease during the Continuous treatment period, because of the patient's health status, etc., administration of the investigational product at the subject's home (self-administration) may be performed by subjects or their families trained in the administration of the investigational product in addition to the administration at the subject's home or nearby medical institutions. In the considerations for storing the investigational products at the subject's house (see "5.6.2.1. During the Treatment Period"), it is not required to lock if it is self-administered. If the investigational products are stored at the subject's home, the maximum number of investigational drugs that can be prescribed is 28.

If the effects of the new coronavirus infection force us to take measures that differ from the provisions of the study protocol and standard procedures, we shall follow the "Procedures for Conducting a Clinical Trial under the Influence of a New Coronavirus Infection," which is provided separately, while placing the highest priority on ensuring the safety of subjects. In providing the investigational drug for administration at the subject's home, the subject may use an investigational drug delivery company to have the medication delivered directly to his/her home. With regard to the delivery of the investigational drug to the subject's home, the investigational drug shall be given to the subject without fail in accordance with the "Procedures for Transporting Investigational Drugs to the Subject's Home" separately specified, with due attention to personal information management.

2702