

# Ropinirole hydrochloride remedy for amyotrophic lateral sclerosis - Protocol for a randomized, double-blind, placebo-controlled, single-center, and open-label continuation phase I/IIa clinical trial (ROPALS trial)

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## Method Article

**Keywords:** Amyotrophic Lateral Sclerosis, Ropinirole hydrochloride, Requip CR, iPSC-drug discovery

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## Abstract

**Background:** Amyotrophic lateral sclerosis (ALS) is one of intractable neurological diseases, which is an incurable disease. ALS is a progressive disease characterized by muscle atrophy and weakness caused by selective vulnerability of upper and lower motor neurons. In the disease research ever conducted, it has been common to use mouse models carrying mutations in responsible genes for familial ALS as pathological models of ALS. However, there is no model that has reproduced the actual conditions of human spinal cord pathology. Thus, we developed a method of producing human spinal motor neurons using human induced pluripotent stem cells (iPSCs) and an innovative experimental technique for drug screening. As a result, ropinirole hydrochloride was eventually discovered after considering preferable transitivity in the brain, tolerability including adverse reactions, and others. Therefore, we explore the safety, tolerability and efficacy of ropinirole hydrochloride to ALS in this clinical trial.

**Methods/Design:** ROPALS trial is a single-center double-blind randomized parallel group-controlled trial of the safety, tolerability, and efficacy of ropinirole hydrochloride extended-release tablet (Requip CR) at 2mg - 16mg doses in patients with ALS. 20 patients will be recruited as active drug and placebo group. All patients will be able to be on the standard treatment for ALS of riluzole if not changed the dosage. The primary outcome will be safety and tolerability at 24 weeks defined from the date of randomization. Secondary outcome will be the efficacy including the change of ALS Functional Rating Scale-Revised (ALSFRRS-R), the change in Combined Assessment of Function and Survival (CAFS), and composite endpoint as a sum of Z-transformed scores on the various clinical items. Noteworthy, we will perform explorative search for drug effect evaluation by using the patients-derived iPSCs to prove this trial concept. Eligible patients will have El Escorial Possible, clinically possible and laboratory-supported, clinically probable, or clinically definite amyotrophic lateral sclerosis with disease duration less than 60 months (inclusive), ALSFRRS-R score  $\geq 2$  points on all items and age from 20 to 80 years.

**Discussion:** Patient recruitment began in December 2018 and the last patient is expected to complete the trial protocol in November 2020.

**Trial registration:** Current controlled trials UMIN000034954

Protocol version: version 1.1 (Date; 7/Nov/2018)

**Keywords:** Amyotrophic Lateral Sclerosis, Ropinirole hydrochloride, Requip CR, iPSC-drug discovery

## Background

Amyotrophic lateral sclerosis (ALS) is one of intractable neurological diseases, which is an incurable disease. ALS is a progressive disease characterized by muscle atrophy and weakness caused by selective vulnerability of upper and lower motor neurons. Patients with ALS develop symptoms such as gait difficulty, language disorder, dysphagia, and respiratory disorder, which confound the freedom of their lives and communication, whereas their consciousness and perception are completely normal, and this feature of the disease significantly reduces their quality of life (QOL). [1]

The annual crude prevalence and incidence rates per 100 000 people per year were 9.9 (95% CI 9.7–10.1) and 2.2 (95% CI 2.1–2.3), respectively in Japan. The male-female ratio was approximately 1.5 and the age group with the highest prevalence as well as incidence was 70–79 years [2]. ALS develops mainly after the middle-age period of their lives, which impedes their returning to society. Therefore, psychological and financial burdens of not only patients but also their family are serious. While clinical course varies among patients, the median time from onset to death or to use of respiratory support has been reported to be 20 to 48 months.

Familial ALS (FALS) accounts for 5% to 10% of all ALS cases, and the other 90% are classified as sporadic ALS (SALS), which has not been clearly elucidated for its genetic background or etiologic factors. More than 100 point mutations spanning the SOD1 sequence have been identified in patients with FALS (gain of function type). Besides, responsible genes have also been reported at least 25 [1]. Therefore, in order to develop treatment options for ALS including SALS that occupies the majority of ALS, an approach to pathological conditions that are common to FALS and SALS is required. Specific loss and degeneration of upper and lower motor neurons and their nerve fibers are present in both the pathological conditions of FALS and SALS, and how to prevent motor neuron degeneration and death is the key in developing treatment options that are common to both forms of ALS.

The pathological conditions of ALS have been studied, and the cellular process that follows after its onset, more specifically, mitochondrial dysfunction, protein aggregation, oxidative stress, agitation toxicity, inflammatory reaction, and apoptosis, has been partly elucidated. Mitochondrial abnormalities can occur as the initial event of neurodegeneration or secondary to other cellular process, and may also be the cause of oxidative stress, agitation toxicity, and apoptosis in some cases [1].

Riluzole, which is considered to exert neuroprotective effects by reducing glutamate toxicity, has been approved for the treatment of ALS. Riluzole was shown to potentially increase survival in an overseas clinical study, and is therefore used widely in Japan [3-8]. However, the effect of riluzole is not fully satisfactory for patients. Also, edaravone injection solution was approved for an additional indication of inhibition of the progression of functional disorder in ALS. However, no study has been conducted to confirm the impact on survival, and the beneficial effect on survival has not yet been verified [9-10]. Under these circumstances, development of treatment options to promote motor neuron survival is anticipated.

Ropinirole hydrochloride (trade name: Requip Tablets 0.25 mg, 1 mg, and 2 mg) is a dopamine receptor agonist with a non-ergot alkaloid chemical structure, which was synthesized and developed based on the structure of dopamine by GlaxoSmithKline UK Limited Company. Ropinirole hydrochloride is selective for the D<sub>2</sub> subtype of dopamine receptors. Ropinirole hydrochloride was first approved for the indication of Parkinson's disease in the UK in July 1996 and has been approved worldwide. The drug in this trial (Requip Controlled-release (CR) Tablets 2 mg and CR Tablets 8 mg) is an extended-release formulation of ropinirole hydrochloride. This product was first approved in the Slovak Republic in 2006 and has been approved worldwide.

Ropinirole hydrochloride has been demonstrated to not only improve motor symptoms of Parkinson's disease by stimulating the dopamine receptors, but also, display the following neuroprotective properties in animal models: 1) Effect to prevent the decrease in 6-OHDA-induced striatal dopamine levels [11], 2) Effect to increase glutathione, SOD, and catalase activities,[11-12], 3) Effect to promote neurotrophic factor production in the ventral mesencephalon [13] and 4) Effect to promote neural stem cell proliferation in the subventricular zone [14].

Pramipexole hydrochloride with the activity as dopamine receptor agonist, which is an analog of ropinirole hydrochloride, has been demonstrated to have the protective effect on mitochondria as well as the free radical scavenging effect. Therefore, with hope for improving pathological conditions of ALS, a clinical study was conducted (R(+)) enantiomer of pramipexole [dextramipexole; RPPX] without the activity as dopamine receptor agonist was actually used in the study due to concerns for adverse drug reactions [ADRs]). A phase I clinical study of RPPX was conducted as a randomized, double-blind, placebo-controlled study in 54 healthy volunteers; RPPX was demonstrated to be well tolerated at doses up to 300 mg/day [15]. In the following phase II study in 30 ALS patients, RPPX was administered at a dose of 30 mg/day for 6 months; RPPX was demonstrated to be tolerated and also to improve the slope of decline on the ALS Functional Rating Scale-Revised (ALSFRS-R) scale by 13% [16]. In a dose escalation study in 10 ALS patients, the dose of RPPX was increased to a maximum of 300 mg/day, which was confirmed to be safe and tolerable with no dopaminergic ADRs reported. This study was continued as an extension study, in which RPPX was administered at doses of 30 mg/day and 60 mg/day for 6 months for a comparison purpose. As a result, the decline (exacerbation) of the slope of ALSFRS-R score was smaller at 60 mg/day than at 30 mg/day [16]. Next, a randomized, double-blind, placebo-controlled, phase II study was conducted, and the safety and tolerability of RPPX were evaluated in ALS patients. This study was divided into two parts: at Stage 1, 102 subjects were randomized to receive one of RPPX 50 mg/day, 150 mg/day, 300 mg/day, and placebo for 12 weeks. At Stage 2, 92 subjects who underwent a 4-week washout were randomized to receive either 50 mg/day or 300 mg/day for 24 weeks. RPPX was generally safe and well tolerated. The slope of ALSFRS-R score was markedly reduced in the higher dose group at both Stages 1 and 2, and the hazard ratio of mortality was reduced by 68% in the 300 mg/day group, compared with the 50 mg/day group, at Stage 2 (p=0.07, log-rank test). Treatment at 300 mg/day was significantly more beneficial in terms of the integrated outcome of the changes in ALSFRS-R and mortality (p=0.046, joint-rank test) [17].

Based on these results, a phase III, multicenter, randomized, double-blind, placebo-controlled study of RPPX was conducted in ALS patients in the US, Canada, Australia, and Europe; however, regrettably, the results turned out to be clinically insignificant [18]. Nevertheless, there is still plenty room for improvement in clinical study design including selection of patients, treatment method, and evaluation methods (especially, those other than ALSFRS-R).

In the disease research ever conducted, it has been common to use mouse models carrying mutations in responsible genes for ALS as pathological models of ALS. However, there is no model that has reproduced the actual conditions of human spinal cord

disturbance, except for mutant SOD-1 transgenic model. It is also known that aggregation of phosphorylated TDP-43, which is known as the most major abnormally aggregated protein in ALS, is not found even in the mutant SOD-1 model that is frequently used overseas. Thus, we developed a method of producing human spinal motor neurons using human iPSCs and an innovative experimental technique for drug screening [19]. Using this system, spinal motor neurons were produced from iPSCs from healthy individuals as well as patients of familial ALS (TDP-43 and FUS mutations) and/or SALS, and drug screening was then carried out using existing drug libraries with the improvement in the ALS-related phenotypes in patients-derived spinal motor neurons in a dish. As a result, several candidate drugs came up, and ropinirole hydrochloride was eventually discovered after considering the blood brain barrier permeability, tolerability including ADRs, and others.

Therefore, we explore the safety, tolerability and efficacy of ropinirole hydrochloride to ALS in this clinical trial.

## Methods

### 1. Study Objectives

*Primary Objective:* To exploratively assess the safety (type, frequency, and severity of adverse events (AEs), and time course of laboratory test values) and tolerability of the ropinirole hydrochloride extended-release tablet in ALS patient after oral treatment with the ropinirole hydrochloride extended-release tablet in ALS patients.

*Secondary Objective:* To exploratively assess the efficacy of the ropinirole hydrochloride extended-release tablet, compared with placebo, in terms of delay in the progression of ALS.

### 2. Subject Population

Patients affected by probable (clinically or laboratory supported) or definite ALS [20] must satisfy all the inclusion and exclusion criteria (Table 1) upon the interim registration during the 28-days screening period through clinical evaluation and laboratory and instrumental assessment. Screening assessments include general and neurological examinations, ALSFRS-R, blood sampling, biochemical and pregnancy evaluations (for fertile females), urinalysis, ECG, and spirometry. Moreover, patients also must comply with all the inclusion and exclusion criteria (Table 1) upon the official registration after the 3-months run-in period.

### 3. Explanation to Subjects and Caregivers and Their Informed Consent

#### 3.1 Subjects

##### 3.1.1 Preparation of Written Information and Informed Consent Form

The investigator will prepare written information for subjects and an informed consent form (hereinafter collectively referred to as the informed consent document). The informed consent document is an all-in-one document or a set of documents, and will be revised, as appropriate. The prepared informed consent document will be submitted to the head of the study site to obtain approval of the IRB prior to the start of the study.

##### 3.1.2 Matters to Be Contained in the Informed Consent Document

Items listed below must be at least contained in the written information for subjects.

- That the study involves research
- The purpose of the study
- The name, title, and contact of the investigator

- The study procedure(s) (including experimental aspects of the study, subject inclusion criteria, and probability for random assignment to each treatment)
- Reasonably expected benefits, and foreseeable risks or inconveniences to subjects (When there is no intended clinical benefit to subjects, the subjects should be made aware of this.)
- Presence/absence of alternative courses of treatment, and if present, their expected notable benefits and risks in a study in patients
- The expected duration of subject's participation in the study
- That subject's participation in the study is voluntary and that the subject can withdraw from or refuse participation in the study or his/her legal representative can withdraw the subject from or refuse his/her participation in the study at any time, without penalty or loss of benefits to which the subject is otherwise entitled
- That individuals involved in the study, including monitors, auditors, IRB, etc. and regulatory authorities, may request direct access to source documents, without violating the subject's confidentiality, and that, by signing or sealing the informed consent form, the subject or his/her legal representative authorizes such access
- That the subject's identity remains confidential even when the study results are published
- The person(s) to contact at the study site for further information about the study and subject's rights or in the event of a study-related health injury
- Compensation and/or treatment available to the subject in the event of a study-related health injury
- The type of the IRB that reviews the appropriateness etc. of the study, items to be reviewed at each IRB meeting, and other IRB-related matters in the study
- The planned number of subjects involved in the study
- That the subject or his/her legal representative will be informed immediately when information is obtained that may affect the subject's or his/her legal representative's willingness to continue participation in the study
- Conditions or reasons for withdrawing the subject from his/her participation in the study
- The anticipated financial burden, if any, on the subject for participation in the study
- The anticipated prorated payment, if any, to the subject for participation in the study (e.g., agreement on payment estimation)
- Matters to be adhered by subjects.

### **3.1.3 Method of Informed Consent Obtainment**

- Prior to the start of the study, the investigator will distribute the informed consent document approved by the IRB to patients as prospective study subjects, and provide them with an adequate explanation about the contents of the study. A study collaborator may provide a supplementary explanation. Explanations should be provided in as plain language as possible, so that patients can understand them, based on the informed consent document for the study, and patient's questions must be adequately answered. After confirming that the patients have fully understood the contents of the explanation, the investigator will obtain their voluntary written informed consent for participation in the study. Interim registration will take place within 28 days of informed consent obtainment.
- The investigator who provides the explanation and the patient will affix their names/seals or signatures to the informed consent form, with the date. The study collaborator who provides a supplementary explanation will also affix his/her name/seal or signature to the informed consent form, with the date.
- If the patient is unable to sign or incapable of reading the informed consent document due to a loss of upper limb function caused by ALS symptoms, the investigator will provide an adequate explanation in the presence of a fair witness, and obtain voluntary written informed consent to participate in the study from the patient. The witness will also affix his/her name/seal or signature to the informed consent form, with the date, and provide the relationship with the patient. If the patient is physically unable to sign, his/her witness will write the subject's name and the reason for his/her inability to give authentic signature to the informed consent form.

- The investigator will issue the signed and dated informed consent document to the subjects before their participation in the study. The original informed consent form will be appropriately retained in accordance with the regulations of the study site.

### **3.1.4 Revision of the Informed Consent Document**

- If new important information that could be relevant to the subject's willingness is obtained, the investigator will immediately decide whether or not to revise the informed consent document based on the obtained information.
- If it is deemed necessary to revise the informed consent document, the investigator must revise the document and forward it to the IRB to reobtain its approval.
- In the case of the above (2), the investigator will immediately notify the subjects already participating in the study of the matter verbally, confirm their willingness to continue participation in the study, and record the result in the medical record.
- The investigator will provide subjects already participating in the study with an explanation using the informed consent document reapproved by the IRB, and obtain voluntary written informed consent for continued participation in the study from the subjects.
- As in the case of obtaining the initial informed consent, the investigator who provides the information and the subject will affix their names/seals or signatures to the informed consent form, with the date. The study collaborator who provides a supplementary explanation will also affix his/her name/seal or signature to the informed consent form, with the date.
- The investigator will issue the signed and dated informed consent document to the subjects. The original informed consent form will be appropriately retained in accordance with the regulations of the study site.

## **3.2 Caregivers**

"Zarit Caregiver Burden Interview" is set as an endpoint in this study. Since this assessment will be conducted by caregivers of the subjects, written informed consent must also be obtained from caregivers. Caregiver's assessment will be made wherever possible, and subjects are able to participate in the study even if their caregivers do not provide informed consent. Subjects will designate their caregivers involved in the assessment. It is allowed to designate two or more caregivers or change the caregivers during the study. If the study for the subject is discontinued, the caregiver's assessment will be ended upon completion of the assessment at the time of discontinuation (wherever possible).

### **3.2.1 Preparation of Written Information and Informed Consent Form**

The investigator will prepare the informed consent document for caregivers. The informed consent document is an all-in-one document or a set of documents, and will be revised, as appropriate. The prepared informed consent document will be submitted to the head of the study site to obtain IRB's approval prior to the start of the study.

### **3.2.2 Matters to Be Contained in the Informed Consent Document**

Items listed below must be at least contained in the informed consent document.

- Qualification required for caregivers involved in the assessment
- Roles of caregivers
- That caregiver's participation in the study is voluntary and that the caregiver can withdraw from or refuse participation in the study at any time, without penalty or loss of benefits to which the subject is otherwise entitled
- Information collected
- Use of study data and protection of privacy
- The name, title, and contact of the investigator

### 3.2.3 Method of Informed Consent Obtainment

- Prior to the start of the study, the investigator will distribute the informed consent document approved by the IRB to caregivers of prospective study subjects, and provide them with an adequate explanation about the contents of the study. A study collaborator may provide a supplementary explanation. Explanations should be provided in as plain language as possible, so that patients can understand them, based on the informed consent document for the study, and caregiver's questions must be adequately answered. After confirming that the caregivers have fully understood the contents of the explanation, the investigator will obtain their voluntary written informed consent for participation in the study.
- The investigator who provides the explanation and the caregiver will affix their names/seals or signatures to the informed consent form, with the date. The study collaborator who provides a supplementary explanation will also affix his/her name/seal or signature to the informed consent form, with the date.
- The investigator will issue the signed and dated informed consent document to the caregivers before their participation in the study. The original informed consent form will be appropriately retained in accordance with the regulations of the study site.

## 4. Study Design

The flow of this study is shown Table 2.

### 4.1 Phase and Type of the Study

Study phase: Phase I/IIa, Study type: Exploratory study

### 4.2 Study Design

- Double-blind period: Single center (Keio university Hospital), randomized, double-blind, placebo-controlled design
- Continued treatment period: Single center (Keio university Hospital), open-label, uncontrolled, active drug continued treatment design

This study consists of the following periods.

- Screening period (from informed consent to interim registration)
- Run-in period: 12 weeks (from interim registration to official registration)
- Double-blind period: 24 weeks
- Tapering treatment period: 0 to 2 weeks
- Continued treatment period (open-label) (only for subjects willing to receive continued treatment): 4 to 22 weeks
- Tapering treatment period (after the continued treatment period): 0 to 2 weeks
- Follow-up period (after the double-blind period, the continued treatment period, or the time of discontinuation): within 28 days

[Screening period]

After obtaining informed consent, necessary tests/observations will be performed. Eligibility assessment will then be conducted, and interim registration will take place. Interim registration will be performed within 28 days of informed consent obtainment.

#### [Run-in period]

After interim registration, eligibility will be reconfirmed during the run-in period (12 weeks  $\pm$  7 days), and official registration will take place. Besides the criteria for interim registration, subjects must have the change in ALSFRS-R score within the range between -2 and -5 points during the 12-week run-in period to be eligible for official registration. This criterion will be confirmed to complete official registration.

#### [Double-blind period]

After the first dose of the study drug, the dose will be increased once weekly. Treatment with the study drug (study treatment) will be started at a first dose of 2 mg, followed by increases in the dose to a maximum of 16 mg, and subjects will be monitored until Week 24. Study treatment will be started within 15 days after official registration. The last dose of study treatment during the double-blind period will be administered on the preceding day of Week 24. If the study proceeds to the continued treatment period, the double-blind period is defined as the period before study drug administration at Week-24. In principle, subjects will be monitored under hospitalization for approximately 1 week from the preceding day of the first dose of study treatment (subjects are allowed to be temporarily discharged during the specified test period if their hospital discharge is considered valid by the investigator). Subsequently, once-weekly dose increase (allowable range:  $\pm$  3 days), treatment, and monitoring will be conducted in outpatient settings.

#### [Tapering treatment period]

After the double-blind period, the dose of the study drug will be tapered in accordance with the Study Drug Tapering Protocol (Table 4). If the study does not proceed to the continued treatment period, the study treatment will be completed.

#### [Continued treatment period]

Upon completion of the double-blind period, the subjects can choose whether to complete the study or continue treatment with the active drug under an open-label design (continued treatment period). The continued treatment period is 4 to 22 weeks; if any of the criteria listed in "12.1 Discontinuation Criteria for Subjects" are met, the study for the relevant subject should be discontinued even before the 22-week period is attained. For subjects who are unable to stay in the study for at least 4 weeks after proceeding to the continued treatment period, the study will be discontinued at the end of the double-blind period without proceeding to the continued treatment period.

When proceeding to the continued treatment period, the dose of the study drug will be tapered (it will take 2 weeks in the case of reducing the dose from the maximum of 16 mg) for both the active drug and placebo groups in accordance with the Study Drug Tapering Protocol (Table 4) to maintain the blindness. Subsequently, treatment with the active drug will be started at a dose of 2 mg, followed by increases in the dose to a maximum of 16 mg in accordance with the Study Drug Titration Protocol (Table 3).

#### [Tapering treatment period (after the continued treatment period)]

After the end of the continued treatment period, the dose of the study drug will be tapered in accordance with the Study Drug Tapering Protocol (Table 4), and the study treatment will be completed.

#### [Follow-up period]

The final observation will be performed within 28 days after the end of the tapering treatment period.

### **4.3 Methods of Blinding, Randomization, etc.**

#### **4.3.1 Method of Blinding**

The study drug randomization manager will confirm the indistinguishability in appearance and packaging form among the ropinirole hydrochloride extended-release 2 mg tablet, the ropinirole hydrochloride extended-release 8 mg tablet, and placebo before

drug assignment.

The study drug randomization manager will prepare the treatment code and emergency code in accordance with the procedural document separately prepared.

#### 4.3.2 Methods of Randomization and Assignment

The investigator will enter the information required for registration in an electronic data capture (EDC) system. Subjects who are eligible for the study will be randomized to either the active drug or placebo group on the EDC system. The result of treatment assignment and the registration number will be transmitted automatically via e-mail to the unblinded pharmacist of the study site.

Subjects will be randomly assigned to either the active drug or placebo at a 3:1 ratio by dynamic allocation incorporating probabilistic elements with the following variables as allocation adjustment factors.

- Number of months after onset (“≥30 months” and “<30 months”)
- Age (“≥ 65 years” and “<65 years”)
- Total ALSFRS-R score (“≤36 points” and “≥37 points”)

#### 4.4 Endpoints

##### 4.4.1 Primary Endpoints

Type, frequency, and severity of AEs, and time course of laboratory test values, and intergroup difference in the proportion of discontinued subjects during the 24-week double-blind period (from official registration to the final observation at Week 24 of the double-blind period).

##### 4.4.2 Secondary Endpoints

- Ratio of change in ALSFRS-R score per 4 weeks between pre-treatment and post-treatment assessments

The change in ALSFRS-R score per 4 weeks during the run-in period and the change in ALSFRS-R score per 4 weeks during the 24-week double-blind period will be calculated, and the latter-to-former ratio will be determined as the delta ( $\Delta$ ) ALSFRS-R ratio. The change in ALSFRS-R score per 4 weeks during the 24-week double-blind period will be calculated using a simple linear regression model with measured ALSFRS-R score as a response variable and the number of days from the start day of treatment at each measurement time point as an explanatory variable.

The ratio between the treatment groups will be tested for comparison.

$$\Delta\text{ALSFRS-R ratio} = \frac{\text{Change in ALSFRS-R score per 4 weeks during the 24-week double-blind period}}{\text{Change in ALSFRS-R score per 4 weeks during the run-in period}}$$

- Intergroup difference in the change in ALSFRS-R score ( $\Delta\text{ALSFRS-R}$ ) during the 24-week double-blind period (from Day 1 to Week 24 of the double-blind period)

ALSFRS-R score will be assessed according to the specified schedule. The difference ( $\Delta\text{ALSFRS-R}$ ) in the change from the day of the first dose of study treatment in ALSFRS-R score at Week 24 of the double-blind period between the treatment groups will be tested for comparison.

- Change in ALSFRS-R score during the continued treatment period (from the assessment at the start to the final assessment of the continued treatment period) and during the overall treatment period (from Day 1 of the double-blind period to the final assessment of the continued treatment period) ( $\Delta$ ALSFRS-R).
- Change in Combined Assessment of Function and Survival (CAFS) score [21] during the 24-week double-blind period (from Day 1 to Week 24 of the double-blind period), during the continued treatment period (from the assessment at the start to the final assessment of the continued treatment period), and during the overall treatment period (from Day 1 of the double-blind period to the final assessment of the continued treatment period).
- Composite endpoint as a sum of Z-transformed scores on the following items [22].
  - ALSFRS-R subscore of each domain (bulbar function, limb function, and respiratory function)
  - ALS severity classification
  - Simple respiratory function test (FEV1, FEV6)
  - Detailed respiratory function test (VC, %FVC, FEV1%)
  - Manual muscle testing (MMT) score (limb and trunk muscles)
  - Quantitative muscle strength (The same muscle for MMT assessment should be used.)
  - Grip strength and pinch strength
  - Modified Norris Scale (Bulbar Symptom Score)
  - Tongue pressure
  - Body weight
  - Amount of physical activity and number of steps
  - Objective muscle mass determined using computed tomography (CT) for skeletal muscle
  - Amyotrophic Lateral Sclerosis Assessment Questionnaire-40 (ALSAQ-40) score (QOL assessment)
- Time to death or time to a specified state of disease progression
  - The time to onset of any of the following events from the day of the first dose of treatment will be assessed.

Death, inability of independent ambulation, loss of unilateral upper limb function, <sup>a)</sup> tracheostomy, respiratory support, <sup>b)</sup> tube feeding, <sup>c)</sup> loss of vocal conversation, <sup>d)</sup> and inability of oral administration. <sup>e)</sup>

1. a) Loss of unilateral upper limb function: A condition where he/she is unable to grip a pen in one hand, as a guide.
2. b) Respiratory support: Noninvasive respiratory support during all-day hours (generally, at least 22 hours except for meal hours) or invasive respiratory support.
3. c) A condition where "Swallowing" on ALSFRS-R is scored 0 points: "Nothing by mouth (NPO); exclusively parenteral or enteral feeding."
4. d) Loss of vocal conversation: Barely speak out to express emotions or unable to speak out.
5. e) The disease progresses for other reasons than the above a) to c), which renders him/her incapable of orally taking the medication.

- Time to %FVC of  $\leq 50\%$

The length of time until %FVC decreases to  $\leq 50\%$  from the day of the first dose of study treatment will be assessed.

- Time to decrease of at least 6 points in ALSFRS-R score (change of at least 6-point decrease in ALSFRS-R score [ $\Delta$ ALSFRS-R] from Day 1 of the double-blind period).

The time to decrease of at least 6 points in ALSFRS-R score (change of at least 6 points [decrease] in ALSFRS-R score [ $\Delta$ ALSFRS-R] from the day of the first dose of study treatment) from ALSFRS-R score measurement on the day of the first dose of the study treatment will be assessed.

- Proportion of patients who discontinued the treatment (discontinuation rate) during the period from Day 1 of the double-blind period to the final assessment of the continued treatment period.

#### 4.4.3 Exploratory Endpoints

- Comparison of *in vitro* drug effect evaluation and clinical effect using patient iPSCs-derived motor neurons

Blood samples will be collected from subjects who have provided separate informed consent and iPSCs will be established at the Department of Physiology, Keio University School of Medicine. These iPSCs will be directed to differentiate into motor neurons to reproduce the pathological conditions of ALS. The cells will then be treated with ropinirole hydrochloride and were assessed for delay in the progression of ALS. The correlation between the results and the change in phenotype of subjects treated with medication will be examined.

- Explorative search of new biomarkers for diagnosis, pathology, and drug effect evaluation
- Measurement of biomarkers related to the ALS pathology, including TDP-43 and NfL in blood and spinal fluid

Proteins such as TDP-43 and NfL, which are biomarkers related to the ALS pathology, in blood and spinal fluid collected from subjects will be measured using single molecule arrays (Simoa™) or immunomagnetic reduction (IMR) assay.

- RNA expression analysis before and after treatment with ropinirole hydrochloride

Total and micro RNAs will be extracted from exosomes in blood and spinal fluid collected from subjects and analyzed using microarrays or RNA-seq. RNA extracts will then be transferred to the Human Genome Center, the Institute of Medical Science, University of Tokyo, for network analysis etc. to identify variable factors associated with disease progression and Hub genes that may contribute to the therapeutic effect of ropinirole hydrochloride.

- Search of known familial ALS genes

Blood samples collected from subjects who have provided informed consent will be transported to the Department of Neurology, Tohoku University School of Medicine, and mutations in known FALS-related genes will be searched using a targeted resequencing panel for screening of ALS.

- Zarit Caregiver Burden Interview (Assessment of Caregivers' Burden) [23, 24]

This assessment will be conducted for subjects who can be assessed by caregivers. Subjects will designate a caregiver involved in the assessment. Subjects are allowed to designate several caregivers but it is preferable to conduct the assessment by the same caregiver as far as possible. Caregivers who are designated as the rater will fill in the “Zarit Caregiver Burden Interview (Assessment of Caregivers’ Burden), and place it in an envelope to keep it out of subjects’ sight, and submit to the investigator.

## **5 Target Sample Size and Study Period**

### **5.1 Target Sample Size and Sample size calculation**

20 subjects for official registration (15 subjects for the active drug group and 5 subjects for the placebo group). The target number of subjects enrolled in this study was set to be 20, taking feasibility into consideration. Considering seriousness of the disease, the ratio of subjects treated with the active drug and placebo is 3:1 (15 subjects : 5 subjects). A summary of biostatistical consideration related to safety assessment for the design of this study is shown below. In this study, the sample size of the placebo group is limited from ethical consideration, and a comparison between the active drug group and the control group will therefore be made in an explorative manner and statistical assessment will be conducted mainly for each treatment group. As for safety assessment, the primary objective of this study, if there exist any clinically significant AE occurring with an incidence of approximately 10% in the active drug group, the scale of this study is enough to detect such an AE with an 80% probability. In other words, clinically significant AEs with relatively low incidences can be detected with a certain probability in this study. As for efficacy assessment, the secondary objective of this study, the change from Day 1 in ALSFRS-R score during the 24-week double-blind period (exacerbation of symptoms) will be assessed as the primary endpoint. In two past confirmatory studies of edaravone in ALS patients [25,26], the weighted mean change in ALSFRS-R score at Week 24 in the placebo group (n = 99 and n = 66) was -6.8 points. Assuming that the true value of the change in ALSFRS-R score and its standard deviation (SD) in the active drug group are -5.5 points, which is similar to the value in the edaravone group, and 6 points, respectively, the probability that the point estimate of the mean change in the active drug group does not exceed the threshold (-6.8 points) is 80% with the sample size of 15 subjects in the active drug group. The efficacy will be exploratively assessed using the point estimate of the mean score and information to plan a next-phase clinical study will be collected.

### **5.2 Study Period**

November 2018 to March 2021

## **6 Study Drug**

### **6.1 Name of Study Drug**

- Test Drug

Name: SK & F101468-A

Generic name: Ropinirole hydrochloride

Dosage form and strength:

SK & F101468-A 2 mg: Each tablet contains 2 mg of ropinirole.

SK & F101468-A 8 mg: Each tablet contains 8 mg of ropinirole.

- Comparator

Name: SK & F101468-A placebo

Dosage form and strength:

SK & F101468-A 2 mg placebo:

Each tablet does not contain ropinirole and is indistinguishable from the SK & F101468-A 2 mg tablet.

SK & F101468-A 8 mg placebo:

Each tablet does not contain ropinirole and is indistinguishable from the SK & F101468-A 8 mg tablet.

## **6.2 Packaging and Labeling of the Study Drug**

- Packaging

Fourteen tablets of the study drug will be packaged in a press-through package (PTP) sheet, and 10 PTP sheets will then be packed in a small box.

- Labeling

Study drug labeling contains the information including a statement of “For clinical study use,” study drug code, manufacturing number, storage method, expiry date, and name, affiliation, title, and address of the sponsor-investigator.

## **6.3 Storage Method**

The study drug should be stored at room temperature.

## **6.4 Methods of Study Drug Handling, Storage, and Management**

The study drug manager will store and manage the study drug in accordance with the “Procedure for Study Drug Management” prepared by the sponsor-investigator. The study drug manager will dispose of unused study drugs after the end of the study.

It is not allowed to use the study drug for other purposes than this study (another clinical study, animal studies, basic experiments, etc.).

## **6.5 Emergency Code Breaking**

If it becomes necessary to urgently identify the study drug for a subject for his/her treatment and safety assurance, the investigator may request the study drug randomization manager to break the emergency code. The detailed procedure for emergency code breaking will be specified in the procedural document separately prepared.

## **7 Treatments Administered to Subjects**

### **7.1 Preparation of the Subject Screening List**

The investigator will prepare a subject screening list, list all subjects who have received an explanation for informed consent, and assign subject identification (ID) codes to subjects who have provided informed consent.

The investigator will manage the registration number and other information of subjects registered (including subjects with discontinuation or suspension of treatment).

## 7.2 Registration of Subjects

- Informed consent to interim registration

The investigator will perform the tests/examinations that are required to assess the eligibility of subjects during the screening period after obtaining informed consent. The investigator will confirm that the subjects satisfy the inclusion and exclusion criteria upon interim registration, and fill in the items that are required for interim registration in the EDC system. Interim registration will take place within 28 days of informed consent obtainment.

- Official registration

The investigator will perform the tests/examinations and observations for the run-in period that are required for official registration, confirm that the subjects satisfy the inclusion and exclusion criteria upon official registration, and fill in the items that are required for official registration in the EDC system. After official registration, the registration number will be automatically assigned by the EDC system. The investigator will confirm that official registration has been completed and then prescribe the study drug. Study treatment will be started within 15 days after official registration.

## 7.3 Dose and Dosage Regimen

- Criteria for administration

[At the time of the first dose]

The study drug will be administered if all of the following conditions are satisfied.

- 1) Events corresponding to Grade  $\geq 3$  of the "Criteria for Seriousness Classification of ADRs, etc. [Notification No. 80 of the Safety Division, Pharmaceutical Affairs Bureau (PAB), dated June 29, 1992]" are not observed in the assessment of the test/examination and observation items, general conditions, and vital signs on the day of administration.
- 2) There is no concern about administration in the assessment of the test/examination and observation items, general conditions, and vital signs on the day of administration.

- Criteria for dose adjustment of the study drug

Study treatment will be started at a dose of 2 mg once daily, followed by increases in weekly increments of 2 mg (up to a maximum of 16 mg) (Table 3). Subjects will be monitored; if a tolerable AE occurs, the dose will be reduced in weekly increments of 2 mg until the AE abates or resolves. The treatment will be continued at the dose that does not involve AEs or discontinued if the AE is intolerable.

## 7.4 Proceeding to the Continued Treatment Period

### 7.4.1 Criteria for Continued Treatment

Subjects who have completed the double-blind period and are willing to receive continued treatment can proceed to the open-label continued active drug treatment period after the double-blind period.

Subjects who satisfy all of the following criteria are eligible for proceeding to the continued treatment period.

- Subjects who are voluntarily willing to receive continued treatment.
- Subjects who do not meet any of the criteria listed in “12.1 Discontinuation Criteria for Subjects.”
- Subjects who can receive study treatment for at least 4 weeks after proceeding to the continued treatment period.
- Subjects who can proceed to the continued treatment period in the judgment of the investigator.

#### **7.4.2 Method of Proceeding to the Continued Treatment Period**

- The investigator will explain the details of the continued treatment period to subjects who satisfy “7.4.1 Criteria for Continued Treatment” by Week 24 of the double-blind period, confirm their willingness, and obtain their written informed consent.
- When proceeding to the continued treatment period, the dose of the study drug will be tapered to 2 mg (it will take 3 weeks in the case of reducing the dose from the maximum of 16 mg) for both the active drug and placebo groups in accordance with the Study Drug Tapering Protocol (Table 4) to maintain the blindness. Subsequently, treatment will be started, followed by increases in the dose to a maximum of 16 mg in accordance with the Study Drug Titration Protocol (Table 3). The period of open-label treatment with the active drug will be extended within the range of a maximum of 48 weeks from the first dose of study treatment. In the continued treatment period, subjects who have been assigned to receive placebo in the double-blind period will be exposed to the active drug and may thus be at a risk for developing AEs. The subjects should therefore be adequately explained this matter before the start of treatment.

#### **7.5 Duration of Treatment**

24 to 50 weeks (including the tapering period)

- Double-blind period: 24 weeks
- Tapering treatment period: 0 to 2 weeks
- Continued treatment period (for subjects willing to receive continued treatment): 4 to 22 weeks
- Tapering treatment period (for subjects proceeding to the continued treatment period): 0 to 2 weeks

#### **7.6 Prohibited and Restricted Concomitant Drugs**

##### **7.6.1 Prohibited Concomitant Drugs**

Concomitant use of CYP1A2 inhibitors (cimetidine, fluvoxamine, etc.), edaravone, and high-dose methylcobalamin (25 mg or 50 mg) is prohibited, regardless of dose and treatment regimen, during the period from interim registration to the end of the study (to the end of continued treatment for subjects who receive continued treatment) or to the time of discontinuation.

##### **7.6.2 Restricted Concomitant Drugs**

Concomitant use of riluzole (brand name: Rilutek Tablets 50 mg or Riluzole Tablets 50 mg “AA”) is allowed during the period from informed consent obtainment to the end of the study (to the end of continued treatment for subjects who receive continued treatment) or to the time of discontinuation. Subjects who are not receiving riluzole before providing informed consent are not allowed to start treatment with riluzole after providing informed consent. Use of riluzole is not a must.

##### **7.6.3 Descriptions about Concomitant Drugs and Therapies**

The investigator or the study collaborator will enter the following information on concomitant drugs and therapies used during the period from informed consent obtainment to the end of the follow-up period or the time of discontinuation into the concomitant drug and therapy pages of the EDC system.

- Concomitant drugs: Name of drug, daily dose, delivery route, duration of treatment, and purpose of use
- Concomitant therapies: Name/content of therapy, duration of use, and purpose of use

## 7.7 Management of Subjects

- Instructions for administration

The investigator, the study collaborator, or the study drug manager (or the person in charge) will provide subjects with instructions for administration, keeping the following in mind.

- 1) Start taking the drug on the day of prescription.
- 2) Take the drug as instructed by the physician.
- 3) Make sure to bring unused drugs (including spare drugs) and empty PTP sheets at the next visit.

- Instructions for lifestyle

The investigator or the study collaborator will provide subjects with instructions for lifestyle, keeping the following in mind.

- 1) Undergo the medical examination and other tests/examinations on the designated days. When the subject cannot make a visit on the scheduled day, make sure to contact the investigator and seek his/her instruction.
- 2) Bring the Clinical Study Participation Card with them and present it when receiving a medical attention at another hospital or at other departments of this hospital. Subjects who are using drugs prescribed by other doctors than the investigator of this study or drugs purchased at pharmacies are required to make sure to inform the investigator or the study collaborator. Subjects who start using an additional drug during the study are also required to make sure to contact the investigator or the study collaborator before its use.
- 3) Try not to modify lifestyle (exercise, meal, etc. in their daily lives) as much as possible.
- 4) Contact study staff if they have something abnormal in their body.
- 5) Use an effective form of birth control (e.g., condom, pill, diaphragm, intrauterine devices (IUD), implantable contraceptives, spermicide) during the study period.
- 6) Do not engage in potentially hazardous activities, including car driving, machine operation, and working in a high place.

- Instructions on how to fill in the dosing diary

The investigator or the study collaborator will distribute a dosing diary to the subjects at the start of study treatment. At this time, the investigator will explain how to fill in the diary and instruct them to fill in the diary every day during the study drug treatment period. The investigator will also instruct them to describe rainy weather information.

- Contact to subject's another attending doctor

The investigator will check whether the subject is receiving other medical attention than that in this study or not. If the subject is receiving a medical attention of another physician, the investigator will contact the relevant physician, with the subject's consent, to inform that the subject is participating in the study. Also, the investigator or the study collaborator will issue the Clinical Study

Participation Card etc. to the subjects and instruct them to present it at another hospital or at other departments of this hospital to inform another physician through the subject that he/she is participating in the study.

## **8 Tests/Examinations and Observations**

### **8.1 Test/Examination and Observation Schedule**

See Table 5.

### **8.2 Test/Examination and Observation Items and Time Points**

#### **8.2.1 Subject Demographics**

The following subject demographic data will be investigated during the screening period.

Age (date of birth), date of informed consent, gender, race, and presence or absence of allergies (drug, food, and others)

#### **8.2.2 Medical History and Concomitant Diseases**

Previous medical history of diseases (previous diseases, including history of surgery; in the past 5 years, in principle; a definite time frame will not be established for the history of cancers and other diseases that may affect the assessment in this study in the judgment of the investigator etc.) and concomitant diseases will be investigated at 12 weeks after interim registration.

Events that occur during the period from after the investigation at 12 weeks after interim registration to the preceding day of the first dose of study treatment will be handled as follows:

- Events that have resolved before the day of the first dose of study treatment: Previous diseases
- Events that are persisting on the day of the first dose of study treatment: Concomitant diseases

#### **8.2.3 Investigations of Concomitant Drugs and Therapies**

Concomitant drugs and therapies that are used during the period from informed consent obtainment to the end or discontinuation of observation will be investigated for the following items.

- Concomitant drugs: Name of drug, dose, delivery route, start date, end date, and reason for use
- Concomitant therapies: Name/content of therapy, start date, end date, and reason for use

#### **8.2.4 Investigation on the Primary Disease**

The primary disease will be investigated for the following items during the screening period.

Classification of ALS (sporadic, familial), previous treatment, time of onset, criteria for the diagnosis of ALS (El Escorial revised, World Congress of Neurosurgery), ALS Severity Classification (Specific Disease Research Survey, Ministry of Health, Labour and Welfare, January 1, 2007), family history (second-degree relatives), and initial symptoms (bulbar paralysis, upper limb symptoms, lower limb symptoms, respiratory muscle paralysis)

### **8.2.5 Height**

Height will be measured during the screening period.

### **8.2.6 Body Weight**

Body weight will be measured during the screening period, at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of study treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, body weight will be measured before study drug administration at each of the following time points, in addition to the above time points. Measurement will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### **8.2.7 Study Treatment Compliance**

Study treatment compliance will be investigated for the following items by checking the dosing diary filled out by the subjects, during the treatment period.

Date of administration, dose, and time of the final administration before each visit day

### **8.2.8 General Conditions**

General conditions (physical findings) will be examined during the period from the start of the screening period to the end or discontinuation of observation.

### **8.2.9 Vital Signs**

- Blood pressure, body temperature, and pulse rate

Measurement will be performed under the same conditions throughout the study period.

Blood pressure, body temperature, and pulse rate will be measured during the screening period, at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), the following day of the first dose of study treatment, before study drug administration at Weeks 2, 3, 5, 9, 13, 17, 21, and 24 after the start of study treatment, during the follow-up period, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, blood pressure, body temperature, and pulse rate will be measured before study drug administration at each of the following time points, in addition to the above time points and during the follow-up period. Measurement will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 26, 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 27, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 28, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

- Respiratory rate

Measurement will be performed under the same conditions throughout the study period.

Respiratory rate will be measured during the screening period, at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), the following day of the first dose of study treatment, before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of study treatment, during the follow-up period, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, respiratory rate will be measured before study drug administration at each of the following time points, in addition to the above time points and during the follow-up period. Measurement will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 26, 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 27, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 28, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### **8.2.10 12-lead Electrocardiography**

Twelve-lead electrocardiography (ECG) will be performed during the screening period, at 12 weeks after interim registration, before study drug administration at Weeks 24 after the start of study treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, 12-lead ECG will be performed before study drug administration at each of the following time points, in addition to the above time points. Measurement will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26 and 47 after the start of study treatment, or the time of termination [Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27 and 48 after the start of study treatment, or the time of termination

### 8.2.11 Screening for Infections

Screening for infections will be performed using serum samples during the screening period and at 12 weeks after interim registration.

HTLV-1 antibody test, HIV antibody test, HBs antigen test, HCV antibody test, and TPHA (only during the screening period)

### 8.2.12 Conventional Laboratory Tests

Blood and urine samples will be collected during the screening period, at 12 weeks after interim registration, the following day of the first dose of study treatment, before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of study treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, blood and urine samples will be collected before study drug administration at each of the following time points, in addition to the above time points. Blood and urine samples will also be collected, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

[Tests]

- Blood biochemistry (under fasting conditions)

Total protein, albumin, BUN, uric acid, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, creatinine, total bilirubin, direct bilirubin, AST (GOT), ALT (GPT), alkaline phosphatase (ALP), LDH, amylase, CPK (CK), Na, K, Cl, Ca, Mg, CRP, blood glucose, hemoglobin Alc (only during the screening period)

- Hematology

White blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, platelet count, morphology, international normalized ratio of prothrombin time (PT-INR), activated partial thromboplastin time (APTT), and fibrinogen

- Urinalysis

pH, specific gravity, protein, glucose, ketone body, occult blood, urobilinogen

### 8.2.13 Specific Laboratory Tests

Blood samples will be collected before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 24 after the start of study treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, blood samples will be collected before study drug administration at the following time points, in addition to the above time points. Blood samples will also be collected, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 48 after the start of study treatment, or the time of termination

[Tests]

(1) Blood biochemistry: Four fractions of fatty acids

(2) Urinalysis: 8-OHdG (CRE-corrected)

### 8.2.14 Blood Ropinirole Concentrations

Blood samples will be collected at 12 weeks after interim registration, Week 2 after the start of study treatment, visits in the following week of dose increase, before study drug administration at Weeks 13 and 24, and the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, blood samples will be collected before study drug administration in the following week of dose increase after the start of study treatment and at each of the following time points, in addition to the above time points. Blood samples will also be collected, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 26, 35, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 27, 36, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 28, 37, and 48 after the start of study treatment, or the time of termination

### 8.2.15 Pregnancy Test (Only for Subjects of Childbearing Potential)

Subjects of childbearing potentials will be confirmed for whether they are pregnant or not by the urine human chorionic gonadotropin (HCG) test during the screening period. The presence of pregnancy will also be confirmed by the serum HCG test at 12 weeks after interim registration. The presence of pregnancy will be further confirmed by the urine HCG test at the time of discontinuation (wherever possible).

For subjects of childbearing potentials who do not proceed to the continued treatment period, the urine HCG test will be performed at Week 24, in addition to the above time points.

For subjects of childbearing potentials who proceed to the continued treatment period, the urine HCG test will be performed at the following time points, in addition to the above time points. The test will also be performed, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 48 after the start of study treatment, or the time of termination

Both urine and serum HCG tests that are specific for beta subunit (HCG- $\beta$ ) will be used in this study. A pregnancy test is not required for men, surgically sterile women, hysterectomized or bilaterally ovariectomized women, and women with at least 1 year elapsing after their last menstruation because the possibility of pregnancy can be ruled out in these subjects.

### 8.2.16 Cerebrospinal Fluid (CSF) Test

CSF test will be performed using lumbar puncture before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 24 after the start of study treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the CSF test will be performed before study drug administration at each of the following time points, in addition to the above time points. The test will also be performed, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 48 after the start of study treatment, or the time of termination

[Tests]

CSF pressure, appearance, cell count, quantitative protein, albumin, quantitative glucose, LDH, Cl, IgG, CRP, and 8-OHdG

### **8.2.17 Ropinirole Concentration in CSF**

CSF samples will be collected using lumbar puncture before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 24 after the start of study treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, spinal fluid samples will be collected before study drug administration at each of the following time points, in addition to the above time points. CSF samples will also be collected, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 48 after the start of study treatment, or the time of termination

### **8.2.18 ALS Functional Rating Scale-Revised**

Assessment by ALSFRS-R will be conducted during the screening period (within 7 days before interim registration), at 4, 8, and 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the assessment by ALSFRS-R will be conducted before study drug administration at each of the following time points, in addition to the above time points. The assessment will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### **8.2.19 Amyotrophic Lateral Sclerosis Severity Classification**

Assessment by ALS severity classification will be conducted during the screening period, at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the assessment by ALS severity classification will be conducted before study drug administration at each of the following time points, in addition to the above time points. The assessment will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### **8.2.20 Simple Respiratory Function Test (FEV1, FEV6)**

The simple respiratory function test will be performed during the screening period, at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the simple respiratory function test will be performed before study drug administration at each of the following time points, in addition to the above time points. The test will also be performed, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### **8.2.21 Detailed Respiratory Function Test (VC, %FVC, FEV1%)**

Detailed respiratory function test will be conducted during the screening period, at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 13 and 24 after the start of treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the detailed respiratory function test will be performed before study drug administration at each of the following time points, in addition to the above time points. The test will also be performed, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 35 and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 36, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 37, and 48 after the start of study treatment, or the time of termination

Blood gas analysis (PaCO<sub>2</sub>, PaO<sub>2</sub>, pH, and HCO<sub>3</sub><sup>-</sup>) will be performed if %FVC is 50% or less after interim registration.

### **8.2.22 Amyotrophic Lateral Sclerosis Assessment Questionnaire-40**

Assessment by ALSAQ-40 [27,28] will be conducted at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the assessment by ALSAQ-40 will be conducted before study drug administration at each of the following time points, in addition to the above time points. The assessment will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### **8.2.23 Neurological Assessment and Quantitative Muscle Strength Measurement**

Muscle strength will be quantitatively determined using an instrument for measuring muscle strength.[29] Neurological assessment will be conducted at 12 weeks after interim registration, before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of study treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the neurological assessment will be conducted before study drug administration at each of the following time points, in addition to the above time points. The assessment will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

#### **8.2.24 Modified Norris Scale (Bulbar Symptom Score)/Tongue Pressure Measurement**

Tongue pressure will be quantitatively determined using an instrument for measuring tongue pressure in addition to the Modified Norris Scale (bulbar symptom score). [30,31] Assessment by the Modified Norris Scale (bulbar symptom score) will be conducted during the screening period, at 12 weeks after interim registration, before study drug administration at Week 5, 9, 13, 17, 21, and 24 after the start of treatment, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the assessment by the Modified Norris Scale (bulbar symptom score) will be conducted before study drug administration at each of the following time points, in addition to the above time points. The assessment will also be conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

#### **8.2.25 Amount of Physical Activity and Number of Steps**

The amount of physical activity and the number of steps in daily living will be quantitatively determined using Active style Pro manufactured and distributed by OMRON Corporation [32]. Prior to the assessment, the rater will check the dosing diary filled out by subjects about rainy weather information. The data will be confirmed at 4, 8, and 12 weeks after interim registration, before study drug administration at Weeks 5, 9, 13, 17, 21, and 24 after the start of study treatment, during the follow-up period, and at the time of discontinuation (wherever possible).

For subjects who proceed to the continued treatment period, the data will be confirmed before study drug administration at each of the following time points and during the follow-up period, in addition to the above time points. The data will also be confirmed, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### **8.2.26 Confirmation of Death, Inability of Independent Ambulation, Loss of Unilateral Upper Limb Function, Tracheostomy, Respiratory Support, Tube Feeding, and Loss of Vocal Conversation**

Whether any of the defined events are present or not will be confirmed before the start of the first dose of study treatment (3 days before, to the day of, the first dose), and during the period from the day of the first dose to the end of observation or the time of discontinuation.

Loss of unilateral upper limb function, respiratory support, tube feeding, and loss of vocal conversation are defined as conditions shown below.

- Loss of unilateral upper limb function: A condition where he/she is unable to grip a pen in one hand, as a guide.
- Respiratory support: Noninvasive respiratory support during all-day hours (generally, at least 22 hours except for meal hours) or invasive respiratory support.
- A condition where "Swallowing" on ALSFRS-R is scored 0 points: "Nothing by mouth (NPO); exclusively parenteral or enteral feeding."
- Loss of vocal conversation: Barely speak out to express emotions or unable to speak out.

### **8.2.27 Skeletal Muscle Computed Tomography**

Skeletal muscle CT scanning will be performed during the screening period, at Week 1 after the start of study treatment (3 days before the first dose to Day 7 after the start of treatment), before study drug administration at Week 24, and at the time of discontinuation (wherever possible).

### **8.2.28 Exploratory Endpoints**

- Comparison of *in vitro* drug effect evaluation and clinical effect using patient iPSCs -derived neurons

Blood samples will be collected from subjects who have provided informed consent for iPSCs production during the period from after interim registration to before the start of the first dose of study treatment (3 days before, to the day of, the first dose). Motor neurons will be induced in iPSCs produced from peripheral blood cells. The motor neurons will be analyzed for (1) confirmation that the ALS pathology has been represented; (2) assessment of disease improvement with treatment with ropinirole hydrochloride; (3) exploration of the action mechanism of ropinirole hydrochloride; and (4) comparison with clinical outcomes [19].

- Explorative search of new biomarkers for diagnosis, pathology, and drug effect evaluation
- Testing of TDP-43, NfL, etc. in blood and CSF

Blood samples will be collected at 12 weeks after interim registration and at Weeks 13 and 24 after the start of study treatment. CSF samples will also be collected at 12 weeks after interim registration and at Week 24 after the start of study treatment. Blood and CSF samples will be collected, wherever possible, at the time of discontinuation.

For subjects who proceed to the continued treatment period, blood and CSF samples will be collected at each of the following time points, in addition to the above time points. Blood and CSF samples will also be collected, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 35 (blood) and 46 after the start of study treatment, or the time of termination (blood and CSF)

[Dose at the end of the double-blind period: 6 mg]

Weeks 36 (blood) and 47 after the start of study treatment, or the time of termination (blood and CSF)

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 37 (blood) and 48 after the start of study treatment, or the time of termination (blood and CSF)

- RNA expression analysis in blood and CSF

Blood samples will be collected from subjects who have provided informed consent for RNA expression analysis at 12 weeks after interim registration and at Weeks 13 and 24 after the start of study treatment. CSF samples will be collected at 12 weeks after interim registration and at Week 24 after the start of study treatment. Blood and CSF samples will be collected, wherever possible, at the time of discontinuation.

For subjects who proceed to the continued treatment period, blood and CSF samples will be collected at each of the following time points, in addition to the above time points. Blood and CSF samples will also be collected, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 35 (blood) and 46 after the start of study treatment, or the time of termination (blood and CSF)

[Dose at the end of the double-blind period: 6 mg]

Weeks 36 (blood) and 47 after the start of study treatment, or the time of termination (blood and CSF)

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 37 (blood) and 48 after the start of study treatment, or the time of termination (blood and CSF)

- Examination of known familial ALS genes

Blood samples will be collected from subjects who have provided informed consent for gene test at 12 weeks after interim registration.

- Zarit Caregiver Burden Interview

Assessment by Zarit Caregiver Burden Interview [23, 24] will be conducted for subjects who can be assessed by caregivers who have provided informed consent for the assessment at 12 weeks after interim registration, before the start of the first dose of study treatment (3 days before, to the day of, the first dose), at Weeks 5, 9, 13, 17, 21, and 24 after the start of study treatment, and at the time of discontinuation (wherever possible). For subjects who proceed to the continued treatment period, the assessment by Zarit Caregiver Burden Interview will be conducted at each of the following time points, in addition to the above time points. The assessment will be also conducted, wherever possible, at the time of discontinuation even after the continued treatment period.

[Dose at the end of the double-blind period: 2 mg to 4 mg]

Weeks 27, 31, 35, 39, 43, and 46 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 6 mg]

Weeks 26, 28, 32, 36, 40, 44, and 47 after the start of study treatment, or the time of termination

[Dose at the end of the double-blind period: 8 mg to 16 mg]

Weeks 27, 29, 33, 37, 41, 45, and 48 after the start of study treatment, or the time of termination

### 8.3 Adverse Events

An AE is any unfavorable and unintended sign (including a laboratory abnormality), symptom, disease or disorder in a subject administered a study drug, whether it is related to the study drug or not. AEs that are attributable to over dosage and improper use are also handled as AEs. Events that occur during the period from the day of the first dose of study treatment to the end of the follow-up period (within 28 days after the end of treatment) are handled as AEs.

The following events are not handled as AEs.

- Weight loss, muscular weakness, arthralgia, myalgia, motor disorder, dyslalia, respiratory disorder, dysphagia, cognitive dysfunction, anxiety disorder, and depression symptoms, which are considered to be symptoms resulting from an exacerbation of the primary disease in the judgment of the investigator
- Procedures performed only for the purpose of testing (e.g., endoscopy)
- Modification of concomitant diseases that is considered to be within the predictable/foreseeable range by the investigator
- Progression of the primary disease that is considered to be within the predictable/foreseeable range
- Absence of unfavorable medical occurrence (e.g., protocol-specified hospitalization, hospitalization already scheduled at the time of informed consent, hospitalization for non-medical but social reasons, hospitalization to enhance the convenience of visits for treatment/testing, etc.)

Changes in laboratory test values that meet any of the following are handled as AEs.

- If any action for study treatment (cessation, discontinuation) becomes necessary due to a change in laboratory test values
- If use of any drug or procedure for treatment becomes necessary due to a change in laboratory test values
- If any surgical intervention has been introduced due to a change in laboratory test values
- If none of the above applies but the change in laboratory test values is an event of medical concern in the judgment of the investigator etc.

### **8.3.1 Action Taken for Adverse Events**

If an AE occurs, the investigator should take appropriate action(s) or treatment. Reported AEs should be followed until the following conditions are attained. AEs that are persisting at the scheduled end day of observation will be followed in the same manner.

- The AE has resolved or is resolving (or stable)
- In the case of sequela, the symptom has become fixed
- The AE has been followed adequately and further follow-up is no longer necessary in the judgement of the investigator

The investigator etc. will enter the details of all the AEs reported, including the date of onset, severity, causal relationship with the study drug, presence/presence of treatment and, if any, the content, and outcome, in the EDC system.

The investigator may seek the opinion of the Independent Data Monitoring Committee about these AEs.

### **8.3.2 Definition of Serious Adverse Events**

A serious adverse event (SAE) is any of the reported AEs that: 1) Results in death, Is life-threatening, 2) Requires inpatient hospitalization or prolongation of the existing hospitalization (except protocol-specified hospitalization, hospitalization already scheduled at the time of informed consent, hospitalization for non-medical but social reasons, hospitalization to enhance the convenience of visits for treatment/testing, etc.), 3) Results in disability/incapacity, 4) May result in disability/incapacity, 5) Is serious according to the above (1) to (5), and 6) Is a congenital anomaly/birth defect in the next generation.

### **8.3.3 Reporting of Serious Adverse Events**

If the information of an SAE that occurs during the period from the day of informed consent to the end of the follow-up period (within 28 days after the end of treatment) is obtained, the investigator will immediately report to the head of the study site and the study drug supplier. Upon receiving a request for provision of further necessary information from the study drug supplier, the head of the study site, or the IRB, the investigator should respond to this. If it becomes necessary to break the emergency code, the investigator will carry out code breaking in accordance with the procedural document. The investigator will report all SAEs to the Independent Data Monitoring Committee. If the relevant SAE needs to be reported immediately to the regulatory authorities, the investigator will report it within the timeframe specified by the regulatory authorities according to the content of the SAE.

### **8.3.4 Assessment of Severity of Adverse Events**

The investigator will assess the severity of all AEs. The severity of AEs will be determined as mild, moderate, or severe using a 3-grade rating scale by the investigator by referring to the grades specified in the "Criteria for Seriousness Classification of ADRs, etc. [Notification No. 80 of the Safety Division, Pharmaceutical Affairs Bureau (PAB), dated June 29, 1992]."

For the events that are not listed in the "Criteria for Seriousness Classification of ADRs, etc. [Notification No. 80 of the Safety Division, Pharmaceutical Affairs Bureau (PAB), dated June 29, 1992]," the investigator will determine the severity by referring to the following criteria.

1) Mild: Easily tolerable without intervention, 2) Moderate: Requires intervention but does not preclude post-treatment tests/examinations or observations, and 3) Severe: Severely interferes with the activities of daily living (ADL).

If the severity of an AE changes during the study period, the highest grade observed during the period will be entered in the EDC system.

### **8.3.5 Assessment of Causal Relationship**

The investigator will assess the causal relationship with the study drug for all AEs in accordance with the following categories.

- Related: The AE resolves after discontinuation of treatment, the AE recurs after resumption of treatment, a statement that the AE could be related to the study drug is provided in the investigator's brochure, there is no confounding risk factor, the AE is consistent with the amount and/or duration of exposure, the potential relationship with concomitant disease(s), etc. is ruled out, etc.
- Unrelated: The reasonable causal relationship between the study drug and the AE is unlikely.

### **8.3.6 Assessment of Outcome**

The outcome of AEs will be assessed on the following 6-grade rating scale.

1) Recovered, 2) Recovering, 3) Not recovered, 4) Recovered with sequelae, 5) Fatal, and 6) Unknown

### **8.3.7 Significant Adverse Events**

Non-specific significant AEs are not defined in this study.

### **8.3.8 Action Taken in the Case that Pregnancy is Found**

Subjects will be explained at the start of the study that they should immediately inform the investigator if any sign of pregnancy is found due to a failure of birth control, e.g., delay in the period for female subjects or male subjects' partners. If a female subject or a male subject's partner is suspected of being pregnant, the investigator should not give study treatment until the potential pregnancy is ruled out based on the pregnancy test result. If a female subject or a male subject's partner is found to be pregnant, the investigator will discontinue the study for the relevant subject and identify the type of the study drug by breaking the code of the study drug. If the drug administered to the subject is the active drug, the investigator will immediately report the matter in writing to the head of the study site, as well as the study drug supplier. The investigator will follow the relevant subject until the completion of delivery or pregnancy. Pregnancy-related SAEs (miscarriage, abortion, birth defect/congenital anomaly) will be handled in accordance with the same procedures as those in "8.3.3 Reporting of Serious Adverse Events."

## **10 Independent Data Monitoring Committee**

The investigator may seek the opinion of the Independent Data Monitoring Committee about the study progress, and evaluation of safety data, as well as efficacy data, if necessary. Even in this case, the sponsor-investigator is responsible for the final decision-making. The responsibilities of the Independent Data Monitoring Committee are shown below.

### **10.1 Safety Monitoring**

- The Committee will examine the details of SAEs reported in this study and conduct risk assessment for the study. The Committee will recommend whether to further continue the study or not, and protocol revisions including change in the inclusion criteria to reduce the risk of AEs, as appropriate.
- For SAEs that are difficult to differentiate from exacerbation of the primary disease among those related to the events defined as "death or a specified state of disease progression" assessed as a secondary endpoint, the risk of these events due to the active drug will be assessed.

### **10.2 Monitoring of the Implementation Status of the Study**

Data related to the implementation status of the study will be monitored to guarantee the quality of this study. The data include the status of subject registration, validity of study subjects, status of withdrawals/dropouts, and protocol compliance status.

## **11 Risks and Benefits for Subjects**

### **11.1 Risks**

- As with other dopamine receptor agonists, sudden onset of sleep and somnolence occurring in the ADL, e.g., when driving, have been reported in patients receiving this product or the ropinirole hydrochloride tablet. Some of these events were associated with accidents. Also, some of the patients who experienced sudden onset of sleep had no warning symptoms such as somnolence beforehand or experienced such events for the first time after 1 or more years had elapsed from the start of treatment with this product.
- Psychiatric symptoms such as hallucination and delusion are considered to be associated with excessive dopamine receptor stimulation [33]. Treatment with dopamine receptor agonists including this product may potentially exacerbate these psychiatric symptoms.

- Dopamine D<sub>2</sub> receptor agonists including this product may cause a decrease in heart rate through inhibition of norepinephrine release from peripheral nerve endings [34].
- In an overseas study, the pharmacokinetics of this product was compared in patients with Parkinson's disease divided into three age groups of <65 years, 65 to 75 years, and >75 years. Oral clearance (CL/F) decreased with increasing age, with a prolonged elimination half-life (T<sub>1/2</sub>) observed [35]. In a Japanese clinical study, the incidence of psychiatric symptoms including hallucination has been reported to be higher in elderly patients (≥65 years) than in non-elderly patients (<65 years).

## 11.2 Benefits

The therapeutic effect of this product for ALS has been confirmed in *in vitro* models. However, whether this product is effective or not in human patients with ALS will be exploratively assessed for the first time in this study. This study therefore does not guarantee the therapeutic effect of this product in treating ALS patients. Nevertheless, when considering that there is no truly effective established approach to the treatment of ALS at present, it is deemed quite meaningful to assess the safety, tolerability, and efficacy of this product.

## 12 Discontinuation Criteria for Subjects and the Procedure

### 12.1 Discontinuation Criteria for Subjects

If any of the following apply, the study will be discontinued.

- Subject's request for discontinuation of the study
- Subject's withdrawal of consent

Subjects who withdraw informed consent for participation in the study while participating in the study will be handled as discontinued subjects.

- Unable to start the first dose of the study drug.
- Marked decrease in respiratory function

If any of the following apply, the study will be discontinued.

- %FVC is ≤50% and PaCO<sub>2</sub> in blood is ≥50 mmHg.
- Tracheostomy is performed.
- Noninvasive respiratory support is required during all-day hours (generally, at least 22 hours except for meal hours) or invasive respiratory support becomes necessary.
- Oral administration of the study drug becomes difficult due to progression of dysphagia.
- Unable to visit the hospital due to progression of ALS.

If the subject becomes unable to visit the hospital to receive the study drug and/or undergo observations due to his/her death or significant progression of disease.

- Unable to visit the hospital twice in a row during the double-blind period or a total 5 times during the study.

These subjects are deemed inappropriate for efficacy assessment and are therefore handled as discontinued subjects.

Even if a similar situation occurs during the continued treatment period, the study for the subject should not be discontinued immediately; if the subject continues to be unable to visit the hospital, the investigator should then determine whether to continue the study for the subject or not.

- If the study drug assigned to a subject is identified by emergency code breaking, the subsequent treatment for the subject should be discontinued.
- Discontinuation based on the decision of the investigator etc.

If any of the following apply, the investigator etc. may discontinue the study.

- 1) The investigator etc. determine that continued study is inappropriate as a result of safety assessment of the study drug based on subject's clinical symptoms, laboratory test values, vital signs, ECGs, etc.
- 2) The investigator etc. determine that the subject is unable to comply with the protocol.
- 3) The investigator determines that the subject is ineligible for the study because he/she is found not to satisfy the inclusion or exclusion criteria after registration, or for other reasons.
- 4) Other circumstances where the study should be discontinued in the judgment of the investigator etc.

Upon decision of discontinuation of the study, the investigator will provide the subject with an explanation about the discontinuation of the study, reason for discontinuation, and required tests/examinations and treatment, etc., and will then proceed with these tests/examinations, treatment, etc. This does not apply to cases where the subject withdraws consent for these tests/examinations etc.

## 12.2 Procedure for Discontinuation

If the study for a subject is discontinued after the start of study treatment, the investigator will take appropriate measures for the relevant subject. The investigator will perform the tests/examinations and observations scheduled at the time of discontinuation, wherever possible. The assessment at the time of discontinuation will be performed within 12 weeks of discontinuation.

The investigator will enter the date of discontinuation, reason for discontinuation, details, background information of discontinuation, action taken, etc. in the EDC system. If the study is discontinued due to AEs, the name of the AE leading to discontinuation will be entered in the discontinuation page of the EDC system. The date of discontinuation is defined as the day when assessment at the time of discontinuation (assessment date) is conducted; however, if the assessment at the time of discontinuation cannot be conducted, the day when discontinuation is determined is handled as the date of discontinuation.

Subjects who have not undergone observations and tests/examinations scheduled at the time of discontinuation or who have no visit after discontinuation will be followed by letter (envelope) or phone to collect information on the reason, subsequent course, etc. The collected information will be entered in the discontinuation page of the EDC system.

The investigator or the study collaborator will make every effort to collect the dosing record of subjects who have no visit after discontinuation by mail and other means.

## 13 Statistical Analysis

Details of statistical analysis will be documented in the Statistical Analysis Plan separately prepared. The Statistical Analysis Plan will be finalized by the time of code breaking.

### 13.1 Analysis Sets

#### 13.1.1 Case Handling

- Efficacy

The following two analysis sets are defined in this study, and analysis will be performed in each of the analysis sets.

## 1) Full Analysis Set (FAS)

The full analysis set (FAS) is an analysis set based on the intention-to-treat (ITT) principle. The FAS is a subset of all subjects enrolled in the study but excludes the subjects listed below.

- Subjects with violation of the eligibility criteria (subjects who failed to satisfy major registration criteria for this study)
- Subjects who have not received any dose of the study drug
- Subjects who have no data at baseline or during the treatment period
- Subjects who withdrew informed consent in the course of the study and refused the use of all of their data

## 2) Per Protocol Set (PPS)

The per protocol set (PPS) is a subset of subjects who are included in efficacy assessment according to the criteria for case handling prepared before data lock. For the endpoints that are measured over time, the case and data inclusion/exclusion criteria will be prepared for each time point.

The FAS is the primary analysis set of efficacy assessment in this study. Statistical analysis in the PPS will be performed only on the change from baseline in ALSFRS-R score at Week 24, which is an important secondary endpoint.

- Safety

The safety analysis set is a subset of subjects who received at least one dose of the study drug.

### 13.1.2 Data Handling

- Imputation of missing data

Imputation of missing data will be performed for efficacy endpoints.

Details of the imputation method and items for imputation will be documented in the Statistical Analysis Plan separately prepared.

- Case handling criteria

The case handling criteria will be determined before data lock.

### 13.2 Demographic and Other Baseline Characteristics

Summary statistics of patient demographic data (age, sex, body weight, etc.) and baseline characteristics will be calculated by treatment group.

### 13.3 Statistical Analysis of Efficacy

The change from baseline (Day 1) in ALSFRS-R score at Week 24 will be analyzed as an important secondary efficacy endpoint. Summary statistics of the measured value and the change from baseline, and the two-sided 95% confidence interval (CI) will be calculated by treatment group. A null hypothesis that the change from baseline at each time point is 0 will be tested by treatment group using a one-sample t-test. The least squares mean will be compared between the treatment groups using contrasts by an

analysis of covariance (ANCOVA) model with baseline value as a covariate. The least squares mean difference and the two-sided 95% CI will be calculated.

For the secondary endpoints listed below, continuous data will be analyzed in the same manner as for the change in ALSFRS-R score. If a significant deviation is found in the distribution of dependent variables, non-parametric approach will be used. For binary data, the point estimate of the ratio will be calculated by treatment group and the two-sided 95% CI for the ratio will be calculated using the Clopper Pearson method. The two-sided 95% CI for the difference in the ratio between the treatment groups will also be calculated using the normal approximation method. For survival time data, Kaplan-Meier plots will be generated and survival function will then be estimated.

The number and proportion of subjects for each maintenance dose (a maximum of 16 mg) will be summarized by treatment group.

## Secondary endpoints

- Ratio of change in ALSFRS-R score per 4 weeks between pre-treatment and post-treatment assessments.
- Change in ALSFRS-R score during the 24-week double-blind period (from Day 1 to Week 24 of the double-blind period) ( $\Delta$ ALSFRS-R).
- Change in ALSFRS-R score during the continued treatment period (from the assessment at the start to the final assessment of the continued treatment period) and during the overall treatment period (from Day 1 of the double-blind period to the final assessment of the continued treatment period) ( $\Delta$ ALSFRS-R).
- Change in CAFS score during the 24-week double-blind period (from Day 1 to Week 24 of the double-blind period), during the continued treatment period (from the assessment at the start to the final assessment of the continued treatment period), and during the overall treatment period (from Day 1 of the double-blind period to the final assessment of the continued treatment period).
- Composite endpoint as a sum of Z-transformed scores on the following items.
  - ALSFRS-R subscore of each domain (bulbar function, limb function, and respiratory function)
  - ALS severity classification
  - Simple respiratory function test (FEV1, FEV6)
  - Detailed respiratory function test (VC, %FVC, FEV1%)
  - MMT score (limb and trunk muscles) and quantitative muscle strength
  - Grip strength and pinch strength
  - Modified Norris Scale (bulbar symptom score)
  - Tongue pressure
  - Body weight
  - Amount of physical activity and number of steps
  - Objective muscle mass determined using CT for skeletal muscle
  - ALSAQ-40 score
- Time to death or time to a specified state of disease progression (defined as any of death, inability of independent ambulation, loss of unilateral upper limb function, tracheostomy, respiratory support, tube feeding, and loss of vocal conversation).
- Time to %FVC of  $\leq 50\%$  (length of time until %FVC decreases to  $\leq 50\%$ ).
- Time to decrease of at least 6 points in ALSFRS-R score (change of at least 6-point decrease in ALSFRS-R score [ $\Delta$ ALSFRS-R] from Day 1 of the double-blind period).
- Proportion of patients who discontinued the treatment (discontinuation rate) during the period from Day 1 of the double-blind period to the final assessment of the continued treatment period.

Furthermore, analysis will be performed on the following exploratory endpoints: (1) comparison of the drug effect using motor neurons derived from patient's iPS cells and clinical effect, (2) explorative search of new biomarkers (changes in TDP-43 and NfL levels in blood and spinal fluid [change in RNA expression in blood and spinal fluid]) for diagnosis, pathology, and evaluation of drug effect, and (3) search of known FALS-related genes.

### **13.4 Statistical Analysis of Safety**

#### **13.4.1 Adverse Events**

The number of AEs and ADRs and the number of subjects with AEs and ADRs will be tabulated by treatment group and the two-sided 95% CI for the incidence will be calculated using the Clopper Pearson method.

#### **13.4.2 Laboratory Test Values and Vital Signs**

For continuous safety variables, summary statistics of the measured value at each time point and change from baseline, and two-sided 95% CI will be calculated by treatment group. For discrete variables, a cross table of data at baseline and each time point will be prepared.

### **13.5 Level of Significance and Multiplicity**

All the analyses in this study will be performed at a two-sided 5% significance level and two-sided 95% confidence level. Efficacy analysis is the secondary objective, and adjustment for multiplicity of tests among the endpoints or time points will not be performed. For safety analysis, statistical power will be prioritized, and adjustment for multiplicity among the endpoints or time points will not be performed.

### **13.6 Primary Analysis**

Data will be locked after the end of the double-blind period of all the subjects but before the end of the continued treatment period of all the subjects, and will then be analyzed.

### **13.7 Deviations from Originally Planned Statistical Analyses**

If any analysis is performed using a different method from that originally specified in the protocol, all changes should be reported in the clinical study report.

## **14 Quality Control and Assurance of the Study**

The sponsor-investigator must conduct "quality control of the study" and "quality assurance of the study" in accordance with the procedural document separately prepared to maintain the quality and reliability of the study. The study site must cooperate for the quality control and assurance of the study by the sponsor-investigator.

In the conduct of quality control of the study, the monitor will confirm that the study is conducted in accordance with the operating procedure for clinical studies prepared by the study site, the latest protocol, and GCP through direct access, as appropriate. The monitor will also confirm that descriptions in the CRF reported by the investigator are accurate and complete and that they are verifiable against study-related records including source documents.

To guarantee that the study is conducted in accordance with the protocol and GCP, the auditor will conduct audits in accordance with the procedural document and confirm that quality control is conducted appropriately.

## **15 Ethics**

### **15.1 Ethical Conduct of the Study**

This study must be conducted in consideration of the ethical principles based on the Declaration of Helsinki, and in adherence to the Pharmaceutical and Medical Device Act (PMD Act), GCP, and protocol.

### **15.2 Institutional Review Board**

The IRB of Keio university hospital reviewed whether to conduct and continue the study or not from the standpoints of ethical, scientific, and medical validity based on the descriptions in the investigator's brochure, protocol, informed consent document, and sample CRF, and approved this trial.

- **Confidentiality of Subjects**

The subject identification code will be used for subject registration and subject identification in the CRF. Personnel involved in this study must protect confidentiality of subjects at times of direct access to source documents for study procedures, publication in medical journals, submission of materials to regulatory authorities, etc.

### **16 Retention of Records etc.**

- Records etc. retained at the study site

The archiving manager designated by the head of the study site will retain study-related documents and records that should be retained at the study site until the date defined in the following 1) or 2), whichever comes later. However, if the sponsor-investigator deems it necessary to retain them for a longer period, the study site will discuss the specific period and method of retention with the sponsor-investigator.

If it is decided that data related to the clinical study results collected in the study are not included in the application dossier, the study drug supplier should notify the head of the study site of the matter and the reason in writing.

1) Date of marketing approval for the study drug (or the date of approval for partial changes in the approved items in the case of additional indications) (date when 3 years have passed since the notification that development of the drug is discontinued or the clinical study results are not included in the application dossier)

2) Date when 3 years have passed since discontinuation or completion of the study

If marketing approval for the study drug is obtained or discontinuation of development is decided due to a failure to obtain approval, the study drug supplier will notify the head of the study site of the matter in writing.

- Records etc. retained by the sponsor-investigator (investigator)

The sponsor-investigator (investigator) will retain study-related documents and records that should be retained by the sponsor-investigator until the date defined in the following 1) or 2), whichever comes later. The sponsor-investigator (investigator) will

discuss the response after the end of the retention period with the study drug supplier.

1) Date of marketing approval for the study drug (or the date of approval for partial changes in the approved items in the case of additional indications) (date when 3 years have passed since the notification that development of the drug is discontinued or the clinical study results are not included in the application dossier)

2) Date when 3 years have passed since discontinuation or completion of the study

If marketing approval for the study drug is obtained or discontinuation of development is decided due to a failure to obtain approval, the study drug supplier will notify the head of the study site of the matter in writing.

## **17 Cost Bearing for the Study**

### **17.1 Financial Source and Conflicts of Interest**

This study will be conducted under the sponsorship of Japan Agency for Medical Research and Development (AMED) (JP 18ek0109329h0001) and K Pharma, Inc. As for the study drug, all test drugs and part of the comparator will be supplied free-of-charge by GlaxoSmithKline K.K. It will be determined at the Conflicts of Interest Management Committee of Keio University that these do not fall under the conflicts of interest acts.

## **18 Compensation for Study-related Health Injuries**

If a subject suffers any study-related health injury, the study site will provide the relevant subject with treatment and other necessary measures.

### **18.1 Medical Care Provision System**

The investigator and the study site will organize sufficient systems that allow for provision of medical care for the treatment of ADRs of the study drug etc. and make every effort to provide the best possible treatment for relevant health injuries.

### **18.2 Purchase of Insurance**

The investigator shall purchase insurance to guarantee the execution of compensation for subject's health injuries and is responsible for compensation according to the regulations on clinical study insurance. The investigator (or subinvestigator) or the study collaborator will distribute written information for compensation to subjects when providing explanations about informed consent for participation in the study.

## **19 Study Registration**

This study will be registered to one of the databases listed below before the start of the study (before the start of case registration).

- Database established by the National University Hospital Council of Japan (University hospital Medical Information Network [UMIN])
- Database established by the Japan Medical Association

## **20 Protocol Compliance, Deviation, or Change**

### **20.1 Protocol Compliance**

The investigator must comply with this protocol.

### **20.2 Protocol Deviation or Modification**

The investigator must not deviate from the protocol or modify the protocol without written approval based on the IRB's prior review. However, the investigator is allowed to do this in unavoidable medical situations, including the case where such an action is required to avoid an urgent risk to the subject, without prior approval of the IRB.

In such a case, the investigator confirms that the content of and reason for deviation or modification and subsequent protocol revision are appropriate, and submit the draft to the head of the study site and the IRB as soon as possible to gain its approval. The agreement of the head of the study site is also required.

The investigator must record all protocol deviations. Only for protocol deviations that arise in unavoidable medical situations including the case where such an action is required to avoid an urgent risk to subjects, the investigator will prepare a written document to explain the deviation and the reason, and immediately submit it to the head of the study site. The investigator (or subinvestigator) will retain a copy of the document.

The investigator will immediately submit the report on all changes in the study procedures that may significantly affect the conduct of the study or increase the risk to subjects to the head of the study site and the IRB.

## **21 Protocol Revision**

If it is deemed necessary to modify the protocol in the course of the study, the sponsor-investigator will revise the protocol. The sponsor-investigator will immediately notify the head of the study site of the content of the revision in writing, and obtain the IRB's approval through the head of the study site.

If a revision of the protocol is instructed from the head of the study site based on the IRB's opinion, the sponsor-investigator will decide whether the changes are valid or not and revise the protocol, if necessary. The sponsor-investigator will immediately notify the head of the study site of the content of the revision in writing, and obtain the IRB's approval through the head of the study site.

## **22 Ownership and Publication of Results**

Intellectual property rights etc. arising from this study shall belong to researchers. Researchers and drug suppliers shall use part or all of clinical study results for the purpose of application for marketing approval of the study drug. In doing this, the clinical study results are partially disclosed in accordance with applicable laws and regulations; however, subject's personal information remains protected.

## **Discussion**

We believe that this study is proof of concept for iPSC-drug discovery if ropinirole hydrochloride is effective in ALS patients. Patient recruitment began in Dec 2018 and the last patient is expected to complete the trial protocol in November 2020.

## **Abbreviations**

ADR = Adverse reaction, AE = Adverse effect, ALP = Alkaline phosphatase, ALS = Amyotrophic lateral sclerosis, ALT = Alanine aminotransferase, ALSFRS-R = ALS Functional Rating Scale-Revised, ALSAQ-40 = Amyotrophic Lateral Sclerosis Assessment Questionnaire-40, APTT = Activated partial thromboplastin time, AST = Aspartate aminotransferase, BUN = Blood urea nitrogen, CAFS = Combined Assessment of Function and Survival, CK = Creatine kinase, CPK = Creatine phosphokinase, CRP = C-reactive protein, CTCAE = Common terminology Criteria for Adverse Events, EDC = Electronic data capture, FALS = Familial ALS, FAS = Full analysis set, %FVC = Forced vital capacity, GCP = Good clinical practice, HBs = Hepatitis B surface, HCG = Human chorionic gonadotropin, HCV = Hepatitis C virus, HDL = High-density lipoprotein, HIV = Human immunodeficiency virus, HTLV-1 = Human T-cell leukemia virus type 1, IRB = Institutional review board, LDH = Lactate dehydrogenase, LDL = Low-density lipoprotein, MMT = Manual muscle testing, NfL = Neurofilament light chain, PPS = Per protocol set, PT = Prothrombin time, QOL = Quality of life, SAE = Severe adverse effect, SALS = sporadic ALS, SOD = Superoxide dismutase, TDP-43 = Transactive response DNA-binding protein 43, TPHA = Tereponema pallidum hemagglutination, 6-OHDA = 6-hydroxydopamine, 8-OHdG = 8-Hydroxydeoxyguanosine

## Declarations

We believe that this study is proof of concept for iPSC-drug discovery if ropinirole hydrochloride is effective in ALS patients. Patient recruitment began in Dec 2018 and the last patient is expected to complete the trial protocol in November 2020.

### Declarations

#### Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Keio university based on the Helsinki declaration. Written informed consent is obtained from the all participants of this trial.

#### Consent for publication

Not applicable.

#### Availability of data and material

Not applicable.

#### Competing interests

K.F. is a President of K Pharma Inc. H.O. is a Scientific Advisory Board of K Pharma Inc.

#### Funding

This study will be conducted under the sponsorship of Japan Agency for Medical Research and Development (AMED) (JP 18ek0109329h0001) and K Pharma, Inc. As for the study drug, all test drugs and part of the comparator will be supplied free-of-charge by GlaxoSmithKline K.K. It will be determined at the Conflicts of Interest Management Committee of Keio University that these do not fall under the conflicts of interest acts.

## Authors' contributions

S.M. and S.T. were the Sub Investigator and wrote the protocol.

J.N. are Principal Investigators for the trial.

K.F., H.S., N.S., M.A. contributed to the protocol.

H.O. contributed to the protocol and supervised the trial management.

All authors sit on the Trial Management Group.

All authors contributed to the manuscript.

All authors read and approved the final manuscript.

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## References

- [1] Brown RH, Al-Chalabi A; Amyotrophic Lateral Sclerosis. *N Engl J Med* 2017, 13;377(2):162-172
- [2] Doi Y, Atsuta N, Sobue G, Morita M, and Nakano I, for Research Committee of CNS Degenerative Diseases of Japan; Prevalence and Incidence of Amyotrophic Lateral Sclerosis in Japan *J Epidemiol* 2014;24(6):494-499.
- [3] Bensimon G, Lacomblez L, Meininger V, the ALS/Riluzole Study Group; A controlled trial of riluzole in amyotrophic lateral sclerosis. *N Engl J Med* 1994, 3;330(9): 585-591.
- [4] Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V.; Doseranging study of riluzole in amyotrophic lateral sclerosis. *Lancet* 1996, 25;347(9013):1425-1431.
- [5] Lacomblez L, Bensimon G, Leigh PN, Guillet P, Powe L, Durrleman S, Delumeau JC, Meininger V; A confirmatory dose-ranging study of riluzole in ALS. ALS/Riluzole Study Group-II. *Neurology* 1996;47(6 Suppl 4):S242-250.
- [6] Pongratz D, Neundörfer B, Fischer W; German open label trial of riluzole 50 mg b.i.d. in treatment of amyotrophic lateral sclerosis (ALS). *J Neurol Sci* 2000, 1;180(1-2):82-85.
- [7] Riviere M, Meininger V, Zeisser P, Munsat T; An analysis of extended survival in patients with amyotrophic lateral sclerosis treated with riluzole. *Arch Neurol* 1998;55(4):526-528.
- [8] Yanagisawa N, Tashiro K, Tohgi H, Mizuno Y, Kowa H, Kimura J, Takahashi K, Goto I, Ohashi Y. Efficacy and safety of riluzole in patients with amyotrophic lateral sclerosis: double-blind placebo-controlled study in Japan. *Igakuno Ayumi* 1997; 182:851–866.
- [9] Yoshino H, Kimura A; Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis (Phase II study). *Amyotroph Lateral Scler* 2006;7(4):241-245.

- [10] Abe K, Itoyama Y, Sobue G, Tsuji S, Aoki M, Doyu M, Hamada C, Kondo K, Yoneoka T, Akimoto M, Yoshino H; Edaravone ALS Study Group; Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients. *Amyotroph Lateral Scler Frontotemporal Degener* 2014;15(7-8):610-617.
- [11] Iida M, Miyazaki I, Tanaka K, Kabuto H, Iwata-Ichikawa E, Ogawa N; Dopamine D2 receptor-mediated antioxidant and neuroprotective effects of ropinirole, a dopamine agonist. *Brain Res* 1999, 14;838(1-2):51-59.
- [12] Tanaka K, Miyazaki I, Fujita N, Haque ME, Asanuma M, Ogawa N; Molecular mechanism in activation of glutathione system by ropinirole, a selective dopamine D2 agonist. *Neurochem Res* 2001;26(1):31-36.
- [13] Du F, Li R, Huang Y, Li X, Le W; Dopamine D3 receptor-preferring agonists induce neurotrophic effects on mesencephalic dopamine neurons. *Eur J Neurosci* 2005;22(10):2422-2430.
- [14] Höglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, Hirsch EC; Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nat Neurosci* 2004;7(7):726-735.
- [15] Bozik ME, Mather JL, Kramer WG, Gribkoff VK, Ingersoll EW; Safety, tolerability, and pharmacokinetics of KNS-760704 (dexpramipexole) in healthy adult subjects. *J Clin Pharmacol* 2011;51(8):1177-1185.
- [16] Wang H, Larriviere KS, Keller KE, Ware KA, Burns TM, Conaway MA, Lacomis D, Pattee GL, Phillips LH 2nd, Solenski NJ, Zivkovic SA, Bennett JP Jr; R+ pramipexole as a mitochondrially focused neuroprotectant: initial early phase studies in ALS. *Amyotroph Lateral Scler* 2008 ;9(1):50-58.
- [17] Cudkowicz M, Bozik ME, Ingersoll EW, Miller R, Mitsumoto H, Shefner J, Moore DH, Schoenfeld D, Mather JL, Archibald D, Sullivan M, Amburgey C, Moritz J, Gribkoff VK; The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. *Nat Med* 2011, 20;17(12):1652-1656.
- [18] Cudkowicz ME, van den Berg LH, Shefner JM, Mitsumoto H, Mora JS, Ludolph A, Hardiman O, Bozik ME, Ingersoll EW, Archibald D, Meyers AL, Dong Y, Farwell WR, Kerr DA; EMPOWER investigators; Dexpramipexole versus placebo for patients with amyotrophic lateral sclerosis (EMPOWER): a randomised, double-blind, phase 3 trial. *Lancet Neurol* 2013;12(11):1059-1067.
- [19] Fujimori K, Ishikawa M, Otomo A, Atsuta N, Nakamura R, Akiyama T, Hadano S, Aoki M, Saya H, Sobue G, Okano H; Modeling sporadic ALS in iPSC-derived motor neurons identifies a potential therapeutic agent. *Nat Med* 2018;24(10):1579-1589.
- [20] Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases.; El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1(5):293-299.
- [21] Berry JD, Miller R, Moore DH, Cudkowicz ME, van den Berg LH, Kerr DA, Dong Y, Ingersoll EW, Archibald D; The Combined Assessment of Function and Survival (CAFS): a new endpoint for ALS clinical trials. *Amyotroph Lateral Scler Frontotemporal Degener* 2013;14(3):162-168.
- [22] Freemantle N, Calvert M, Wood J, Eastaugh J, Griffin C. Composite outcomes in randomized trials: greater precision but with greater uncertainty? *JAMA* 2003, 21;289(19):2554-2559.
- [23] Zarit SH, Reever KE, Bach-Peterson J; Relatives of the impaired elderly: Correlates of feelings of burden. *Gerontologist* 1980;20(6):649-655.
- [24] Zarit SH, Zarit JM; The Memory and Behaviour Problems Checklist 1987R and the Burden Interview. Pennsylvania State University Gerontology Center :University Park, PA. 1990.
- [25] Mitsubishi Tanabe Pharma Co., Ltd : second confirmatory test (in-house material)
- [26] Abe K, Itoyama Y, Sobue G, Tsuji S, Aoki M, Doyu M, Hamada C, Kondo K, Yoneoka T, Akimoto M, Yoshino H; Edaravone ALS Study Group: Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in

amyotrophic lateral sclerosis patients. *Amyotroph Lateral Scler Frontotemporal Degener* 2014;15(7-8):610-617.

[27] Jenkinson C, Levvy G, Fitzpatrick R, Garratt A; The amyotrophic lateral sclerosis assessment questionnaire (ALSAQ-40): tests of data quality, score reliability and response rate in a survey of patients. *J Neurol Sci* 2000, 1;180(1-2):94-100.

[28] Jenkinson C, Fitzpatrick R, Brennan C, Swash M; Evidence for the validity and reliability of the ALS assessment questionnaire: the ALSAQ-40. *Amyotrophic Lateral Scler Other Motor Neuron Disord* 1999, 1(1):33-40.

[29] Shefner JM, Liu D, Leitner ML, Schoenfeld D, Johns DR, Ferguson T, Cudkowicz M; Quantitative strength testing in ALS clinical trials. *Neurology* 2016, 9;87(6):617-624.

[30] Norris FH Jr, Calanchini PR, Fallat RJ, Panchari S, Jewett B; The administration of guanidine in amyotrophic lateral sclerosis. *Neurology* 1974;24(8):721-728.

[31] Hiraoka A, Yoshikawa M, Nakamori M, Hosomi N, Nagasaki T, Mori T, Oda M, Maruyama H, Yoshida M, Izumi Y, Matsumoto M, Tsuga K; Maximum tongue pressure is associated with swallowing dysfunction in ALS patients. *Dysphagia* 2017;32(4):542-547.

[32] Miyamoto S, Minakata Y, Azuma Y, Kawabe K, Ono H, Yanagimoto R, Suruda T; Verification of a motion sensor for evaluating physical activity in COPD patients. *Can Respir J* 2018, 23;2018:8343705.

[33] Wolters EC; Intrinsic and extrinsic psychosis in Parkinson's disease. *J Neurol* 2001, 248(suppl):22-27.

[34] Acton G, Broom C; A dose rising study of the safety and effects on serum prolactin of SK&F 101468, a novel dopamine D2-receptor agonist. *Br J Clin Pharmacol* 1989;28(4):435-441.

[35] Kaye CM, Nicholls B; Clinical pharmacokinetics of ropinirole. *Clin Pharmacokinet* 2000;39(4):243-254.

## Tables

**Table 1. Inclusion and exclusion criteria for Polaris trial.**

<b>Inclusion criteria</b> [Interim Registration]	<b>Exclusion criteria</b>
1) Patients who have a diagnosis of “clinically possible and laboratory-supported ALS,” “clinically probable ALS,” or “clinically definite ALS” according to the criteria for the diagnosis of ALS (El Escorial revised, World Congress of Neurosurgery) and who are within 60 months after onset of the disease. 2) Grade 1 or 2 according to the ALS Severity Classification (Specific Disease Research Survey, Ministry of Health, Labor and Welfare, January 1, 2007). 3) Japanese patients between 20 and 80 years of age at the time of informed consent. 4) ALSFRS-R score $\geq 2$ points on all items (“Handwriting” and “Eating motion (1)” should be scored $\geq 2$ points on each side). 5) Forced vital capacity (%FVC) $\geq 70\%$ . 6) Written informed consent for participation in the study provided by themselves. 7) Able to be treated in outpatient settings (partially under hospitalization) during the study.	1) Patients who have serious hepatic disorder, renal disorder, cardiac disease, pulmonary disease, blood disorder, metabolic disease, etc., and are considered ineligible for the study by the investigator (Grade $\geq 3$ of the “Criteria for Seriousness Classification of Adverse Drug Reactions, etc. [Notification No. 80 of the Safety Division, Pharmaceutical Affairs Bureau (PAB), dated June 29, 1992]” should be used for reference). 2) Have participated in another clinical study or interventional clinical research within 30 days before interim registration. However, participation in “Establishment of induced Pluripotent Stem Cells (iPSCs) from Patients with Neurological Disease and Disease Analysis using the iPSCs (Approval No. 20080016)” is accepted. 3) Concurrently have cancer or at least 5 years have not elapsed after cure of cancer. 4) Have previously used ropinirole hydrochloride. 5) A family history or prior diagnosis of SOD-1 mutation. 6) Pregnant or potentially pregnant women, or breastfeeding women. 7) Concurrent orthostatic hypotension associated with subjective symptoms (dizziness, syncope, etc.). 8) Concurrent psychiatric disorder or symptoms that are considered to confound their participation in the study. Other individuals who are considered ineligible for participation in this study by the investigator.
[Official Registration]	
8) Change in ALSFRS-R score within the range between -2 and -5 points during the 12-week run-in period. 9) Have not started riluzole treatment, have not reduced the dose of riluzole, or have not discontinued riluzole treatment after the start of the run-in period. 10) Have not used edaravone or high-dose mecobalamin (25 mg or 50 mg) after the start of the run-in period.	
Able to be treated in outpatient settings (partially under hospitalization) during the study.	

Table 2. The flow of this study

Informed consent	Screening period	Interim registration	Run-in period	Official registration	Double-blind period *1	Informed consent for the continued treatment period	Continued treatment period *1	Follow-up period
	28 days after informed consent obtainment		3 months after interim registration		24 weeks		4 to 22 weeks	28 days after the end of treatment
	Eligibility confirmation for interim registration		Eligibility confirmation for official registration		Oral administration of the active drug or placebo	*2	Oral administration of the active drug	Follow-up assessment

\*1: When the study proceeds to the continued treatment period or the treatment is completed, the dose of the study drug should be tapered in accordance with the Study Drug Tapering Protocol (Table 4).

\*2: When the study does not proceed to the continued treatment period, the procedure during the follow-up period should be performed.

Table 3. Study Drug Titration Protocol

After the start of treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	From Week 9
Active drug/ placebo at a dose of 2 mg	1	2	3	4	1	2	3	4	0
Active drug/ placebo at a dose of 8 mg	0	0	0	0	1	1	1	1	2
Total number of tablets taken	1	2	3	4	2	3	4	5	2
Total dose	2 mg	4 mg	6 mg	8 mg	10 mg	12 mg	14 mg	16 mg	16 mg

Table 4. Study Drug Tapering Protocol for Proceeding to the Continued Treatment Period

Final target dose	Week 1	Week 2	Week 3
☐☐ 2 mg	☐ 2 mg (1st week of the continued treatment period)	-	-
☐☐ 4 mg	☐ 2 mg (1st week of the continued treatment period)	-	-
☐☐ 6 mg	☐☐ 4 mg (two 2-mg tablets)	☐ 2 mg (1st week of the continued treatment period)	-
☐☐ 8 mg	☐☐ 6 mg (three 2-mg tablets)	☐☐ 4 mg (two 2-mg tablets)	☐ 2 mg (1st week of the continued treatment period)
☐☐ 10 mg	☐☐ 8 mg (one 8-mg tablet)	☐☐ 4 mg (two 2-mg tablets)	☐ 2 mg (1st week of the continued treatment period)
☐☐ 12 mg	☐☐ 8 mg (one 8-mg tablet)	☐☐ 4 mg (two 2-mg tablets)	☐ 2 mg (1st week of the continued treatment period)
☐☐ 14 mg	☐☐ 10 mg (one 8-mg tablet + one 2-mg tablets)	☐☐ 6 mg (three 2-mg tablets)	☐ 2 mg (1st week of the continued treatment period)
☐☐ 16 mg	☐☐ 12 mg (one 8-mg tablet + two 2-mg tablets)	☐☐ 6 mg (three 2-mg tablets)	☐ 2 mg (1st week of the continued treatment period)

☐: Active drug

☐: Placebo

Due to technical reasons, Table 5 is not displayed

## Supplementary Files

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