STATISTICAL ANALYSIS PLAN

A PHASE 2, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL STUDY TO ASSESS THE EFFICACY, SAFETY, TOLERABILITY, PK AND BIOMARKER EFFECTS OF PTC857 IN ADULT SUBJECTS WITH AMYOTROPHIC LATERAL SCLEROSIS (CARDINALS)

FOR THE
DOUBLE-BLINDED TREATMENT PERIOD (PART A) OF
PTC857-CNS-001-ALS

VERSION 2.0
DATE OF PLAN: 25 SEPTEMBER 2024

STUDY DRUG: UTRELOXASTAT

PROTOCOL NUMBER: PTC857-CNS-001-ALS

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale - Revised
ALS CBS	ALS Cognitive Behavioral Screen
ATC	Anatomical Therapeutic Chemical
BDRM	Blinded Data Review Meeting
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
CAFS	Combined Assessment of Function and Survival
CSF	Cerebrospinal Fluid
CSR	Clinical Study Report
C-SSRS	Columbia-Suicide Severity Rating Scale
DB	Double-Blind
eGFR	Estimated Glomular Filtration Rate
ENR	All Enrolled
ICF	Informed Consent
ITT	Intention to Treat
LFT	Liver Function Test
LTE	Long-term extension
MAR	Missing At Random
MedDRA	Medical Dictionary for Regulatory Activities
NfL	Neurofilament light chain
PAV	Permanent Assisted Ventilation
PFT	Pulmonary Function Test
PK	Pharmacokinetics
PMM	Pattern-Mixture Model
PP	Per Protocol
PT	Preferred Term
SAP	Statistical Analysis Plan
SD	Standard Deviation
SNIP	Sniff Nasal Inspiratory Pressure
SoC	Standard of Care
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
TRICALS	TRICALS is the largest European research initiative to find a cure for ALS
ULN	Upper Limit if Normal
WHO DD	World Health Organization Drug Dictionary

1. INTRODUCTION AND OVERVIEW

The purpose of this statistical analysis plan (SAP) is to provide a description of the statistical methods and procedures to be implemented for the analysis of data from PTC857-CNS-001-ALS study, also referred to as The Cardinals Study. This document details the statistical methods to be used in the analyses and presentation of the data collected during the double-blind treatment period (Part A) of the study. The primary efficacy endpoint of the study is assessed during this period.

The statistical methods to be used for the data collected during the entire study including the open-label long-term extension periods (Parts B and C) will be described in a separate SAP.

This document is based on the final protocol amendment version 6.0, dated 14 JUNE 2024, and has been developed, reviewed, and approved prior to the database lock of the double-blind Treatment Period (Part A). Any changes from the planned analysis as described in the protocol for Study PTC857-CNS-001-ALS and its amendments (as applicable) are detailed here, and any differences described here supersede the analysis presented in the protocol. Any additional analyses conducted to supplement the planned analyses and any deviations from the planned analyses described in this SAP will be documented in the Clinical Study Report (CSR).

1.1. Study Design

Brief description of the Study Design

This is a Phase 2, randomized, double-blind, placebo-controlled, parallel-group study to assess the effects of PTC857 (also known as utreloxastat) in adult male and female subjects diagnosed with ALS. Eligible male and female subjects aged between 18 and 80 years will be enrolled in this study. The study consists of 5 periods: Screening, Treatment (Part A), LTE (Part B), Continued LTE (Part C), and Follow Up as illustrated in Figure 1 below.

Approximately 340 subjects who meet the inclusion and exclusion criteria will be randomized in a 2:1 ratio to receive PTC857 (250 mg BID) or matching placebo with 2 stratification factors:

1. Amount of change in ALSFRS-R score during the 8-week Screening Period by points total loss:
    a. <1,
    b. 1-2,
    c. 3-4, or
    d. >4 points total loss
2. Use of edaravone, sodium phenylbutyrate/taurursodiol, or neither for the treatment of ALS at Screening as standard-of-care therapy:
    e. Edaravone
    f. Sodium phenylbutyrate/taurursodiol
    g. Neither edaravone nor sodium phenylbutyrate/taurursodiol

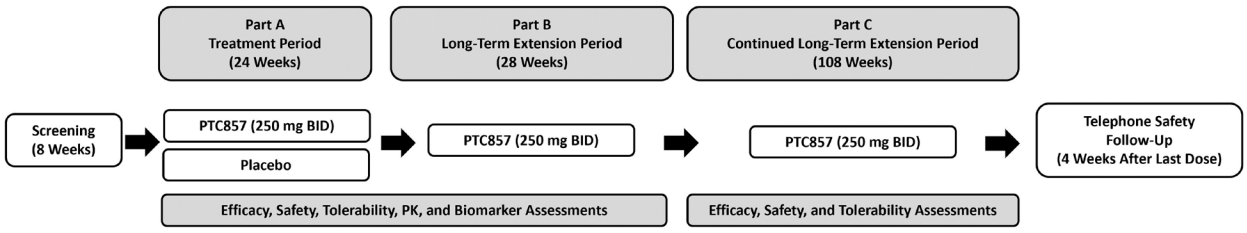
Before protocol amendment version 4.0, dated 31 OCTOBER 2022, the second stratification factor above (treatment for ALS at baseline) was based on use of riluzole alone (with two levels: on riluzole and not on riluzole). Protocol amendment 4.0 opened the study to allow for

randomization of subjects who were taking other treatments for ALS, if approved in their region of participation, specifically edaravone or sodium phenylbutyrate/taurursodiol if their treatment was considered stable. In protocol amendment 4.0, the original riluzole stratification groups (on riluzole vs. not on riluzole) would be amalgamated, and randomization would be based on edaravone, sodium phenylbutyrate/taurursodiol, and Neither treatment status, as indicated above.

After completion of the 24-week double-blind treatment phase, all subjects will be offered the opportunity to enter the optional 28 weeks long-term extension (LTE) Period of the study. Those who choose to enter the LTE Period will sign an additional informed consent form (ICF) and start open-label treatment with PTC857 250 mg BID. Those who choose not to enter the LTE Period will stop treatment, and a telephone follow-up visit will be conducted 4 weeks (±3 days) after the last dose of study treatment. At the end of the LTE Period, those who choose not to enter the Continued LTE Period will stop treatment, and a Telephone Follow-Up Visit will be conducted 4 weeks (±3 days) after the last dose of study treatment. Subjects who enter the Continued LTE Period (Part C) will continue treatment for an optional additional 108 weeks. All subjects will be treated with open-label PTC857 (250 mg BID) during the Continued LTE Period. Subjects will remain blinded to their original treatment from the Treatment Period (Part A). At the end of this period, a Telephone Follow-Up Visit will be conducted 4 weeks (±3 days) after the last dose of study treatment. Results from the Treatment Period (Part A), the LTE Period (Part B), and the Continued LTE Period (Part C) will be reported separately. The scheduled study visit assessments for the double-blind treatment period are presented in Section 11 (Appendix 1).

Total duration of the individual participation will be approximately 172 weeks, including a screening period of 8 weeks mandatory in duration, a double-blind treatment period of 24 weeks, a long-term open-label extension period of 28 weeks, a continued long-term extension period of 108 weeks, and a follow up period of 4 weeks. A subset of approximately 36 subjects from select sites participating in the PK sub-study will participate in serial blood sampling to characterize the PK of PTC857 in subjects with ALS. The study schema is shown below.

Figure 1: Study Design



1.2. Study Objectives

1.2.1. Primary Objectives

The primary objective of this study is to evaluate the efficacy of PTC857 in reducing disease progression in subjects with amyotrophic lateral sclerosis (ALS).

1.2.2. Secondary Objectives

The secondary efficacy objectives of this study are to assess the following in subjects with ALS:

- Safety and tolerability of PTC857
- Respiratory function in subjects randomized to PTC857 versus placebo
- Motor/limb and bulbar function in subjects randomized to PTC857 versus placebo
- Survival in subjects randomized to PTC857 versus placebo
- Quality of life via 40-item Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) in subjects randomized to PTC857 versus placebo
- Evaluate the efficacy of PTC857 in reducing disease progression in subjects with ALS with any baseline rate of functional decline
- Effects on plasma neurofilament light chain (NfL) activity in subjects randomized to PTC857 versus placebo
- Pharmacokinetics (PK) of PTC857

1.2.3. Exploratory Objectives

The exploratory objectives of the study are to assess the following in subjects with ALS:

- Neuropsychological function in subjects randomized to PTC857 versus placebo
- Effects on mechanistic and clinically based biomarker activity in subjects randomized to PTC857 versus placebo
- Quality of life via the five-level European quality of life five-dimensional questionnaire (EQ-5D-5L) in subjects randomized to PTC857 versus placebo
- Exposure-response relationships

1.3. Study Endpoints

1.3.1. Primary Endpoints

The primary endpoint is subject ranks based on the combined assessment of ALS Functional Rating Scale-Revised and survival (CAFS) after 24 weeks of treatment in the Intent-to-Treat 1 (ITT1) Analysis Set.

1.3.2. Secondary Endpoints

The secondary efficacy endpoints are:

- Subject ranks based on the combined assessment of ALSFRS-R and survival (CAFS) after 24 weeks of treatment in the Intent-to-Treat 2 (ITT2) Analysis Set
- Change from baseline in ALSFRS-R in the ITT1 Analysis Set after 24 weeks of treatment.
- Change from baseline in ALSFRS-R in the ITT2 Analysis Set after 24 weeks of treatment.

- Safety and tolerability of PTC857 as measured by the severity and number of treatment-emergent adverse events (TEAEs) and treatment-emergent serious adverse events (TESAEs), and change in clinical laboratory tests, physical examination, vital signs, Columbia-Suicide Severity Rating Scale (C-SSRS), and 12-lead electrocardiograms (ECGs) during the double-blind Treatment Period (Part A)
- Change from baseline in slow vital capacity as assessed by pulmonary function tests (PFTs) after 24 weeks of treatment
- Change from baseline in motor/limb and bulbar function as assessed by the Modified Norris Scale after 24 weeks of treatment
- Survival as assessed by rate of and length of time to death
- Quality of life as assessed by ALSAQ-40 after 24 weeks of treatment
- Change from baseline in plasma NfL activity after 24 weeks of treatment
- Plasma PK and cerebrospinal fluid (CSF) exposure of PTC857

1.3.3. Exploratory Endpoints

The exploratory endpoints of the study are the following:

- Change from baseline in neuropsychological function as assessed by the ALS Cognitive Behavioral Screen (ALS CBS) after 24 weeks of treatment
- Change from baseline in blood, urine, and CSF biomarker activity after 24 weeks of treatment
- Quality of life as assessed by the EQ-5D-5L after 24 weeks of treatment
- Exposure-response relationships after 24 weeks of treatment

1.4. Subject Selection

The study's inclusion and exclusion criteria are detailed in the study protocol.

1.4.1. Inclusion Criteria

Individuals eligible to participate in this study include those who meet all the inclusion criteria at both the Screening Visit and the Baseline Visit, unless otherwise stated:

1. Males or females aged between 18 and 80 years at the time of the initial Screening Visit
2. ALS with preserved function, defined as:
    - Onset of the first symptom leading to the diagnosis of ALS ≤24 months at the time of the initial Screening Visit
    - Revised El Escorial criteria of either:
        i. Clinically definite ALS
        ii. Clinically probable ALS
3. A total ALSFRS-R score of at least 34 at the start of the Screening Period

4. No significant respiratory compromise as evidenced by slow vital capacity ≥60% at the start of the Screening Period
5. Subjects or their designee (i.e., legal authorized representative or caregiver) must understand the nature of the study and must provide signed and dated written informed consent prior to conducting any study-related procedures.
6. Females must be either postmenopausal for ≥1 year (cessation of menses for 12 consecutive months) or surgically sterile (having undergone tubal ligation, hysterectomy, or bilateral oophorectomy) for at least 6 months or, if of childbearing potential and not abstinent, willing to use a highly effective method of contraception from the start of the Screening Period through 90 days after the last dose of study drug. Females who are abstinent will not be required to use a contraceptive method unless they become sexually active.
7. Females must refrain from ova (egg cell) donation from the start of the Screening Period through 90 days after the last dose of study drug.
8. Males, if not surgically sterilized, with female partners of childbearing potential must agree to use barrier contraceptive (i.e., condom) and their female partners must use a highly effective method of contraception from the start of the Screening Period through 90 days after the last dose of study drug.
9. Males must refrain from sperm donations from the start of the Screening Period through 90 days after the last dose of study drug.
10. Willing and able to comply with all protocol procedures.
11. Willing and able to swallow study drug (oral solution) at randomization.
12. All concomitant medications (both prescription and over the counter [OTC]), including standard-of-care therapy riluzole, edaravone, or sodium phenylbutyrate/taurursodiol, and non-pharmacologic therapy regimens should be stable and unchanged from at least 30 days prior to the start of the Screening Period and intend to remain stable and unchanged throughout the course of the study.
13. Female subjects must have a negative breast cancer imaging screening status (not considered clinically abnormal and/or requiring further evaluation/treatment) within 6 months prior to the Screening Visit, or during the Screening Period

1.4.2. Exclusion Criteria

Individuals are not eligible to participate in this study if they have met or meet any of the following exclusion criteria at either the Screening Visit or the Baseline Visit, unless otherwise stated:

1. History of allergies or adverse reactions to any of the excipients in the study drug formulation
2. Females who are pregnant or nursing or plan to become pregnant during the study.
3. Subjects with clinically significant gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, pulmonary, immunologic, psychiatric, or

CV/ischemic disease or any other condition that, in the opinion of the investigator, would jeopardize the safety of the subject or impact the validity of the study results.

4. Any clinically significant medical or psychiatric condition or medical history that, in the opinion of the investigator or the medical monitor, would interfere with the subject's ability to participate in the study or increase the risk of participation for that subject.

   Note: Lumbar punctures will be performed but may be skipped for an individual subject if the investigator deems it appropriate, and after discussion with the medical monitor. Subjects with a contraindication to lumbar punctures, such as but not limited to space-occupying lesion with mass effect, increase of intracranial pressure due to increased CSF pressure, posterior fossa mass, Arnold-Chiari malformation, anticoagulation medication use, coagulopathy, uncorrected bleeding diathesis, congenital spine abnormality, previous adverse event associated with a lumbar puncture or skin infection at the puncture site, should not undergo the lumbar punctures as listed in the Schedule of Events. These subjects may still enroll in the study and should undergo all other study procedures.

5. Hepatic insufficiency, defined as liver function tests (LFTs) (i.e., AST and/or ALT) ≥3× the upper limit of normal (ULN), or bilirubin ≥1.5× the ULN (except in the case of Gilbert's disease)
6. Moderate or worse renal insufficiency, defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min.
7. Current participation in any other investigational study with an investigational product or participation within 30 days prior to the start of the Screening Period or 5 half-lives of the previously taken investigational drug, whichever is longer.
8. History of alcohol or drug abuse within the last 6 months prior to the start of the Screening Period or current evidence of substance dependence
9. Any surgery within 30 days prior to the start of the Screening Period that may affect the subject's ability to complete all study procedures.
10. Subject has previously received PTC857.
11. Subject is receiving a combination of edaravone and sodium phenylbutyrate/taurursodiol treatment, where applicable, within 30 days prior to the start of the Screening Period
12. For female subjects, any past medical history of breast cancer, regardless of remission status, or any first degree relative with history of breast cancer
13. Subjects is taking a non-approved form of sodium phenylbutyrate and/or taurursodiol for the treatment of ALS.

1.5. Sample Size

For the primary efficacy endpoint, the combined assessment of ALSFRS-R and survival after 24 weeks of treatment, 307 subjects in the ITT1 Analysis Set (205 in the PTC857 group and 102 in the placebo group, based on a 2:1 randomization ratio) will provide approximately 85% power to detect a treatment difference of 2 points with a standard deviation of 5.3, based on a 2-sided 0.05 level Joint Rank Test, and assuming 24-week survival rates of 96.5% and 95% in the PTC857 and placebo groups, respectively. These assumptions took into consideration data from 2 clinical

studies in ALS which reached statistical significance for their primary endpoints that reported overall death rates of 0% and 5% (Edaravone (MCl-186) ALS 19 Study Writing Group 2017 and Paganoni, 2020) A standard deviation of 5.3 points for the ALSFRS-R score at Week 24, among treated subjects was reported for the edaravone study (Edaravone (MCl-186) ALS 19 Study Writing Group 2017).

Assuming approximately 10% of randomized subjects will not meet the ITT1 Analysis Set definition, then approximately 340 subjects will be randomized to ensure that 307 subjects are randomized into the ITT1 Analysis Set. Given that the primary analysis incorporates deaths as a combined endpoint, and a fully imputed analysis dataset is utilized in the analysis, additional enrollment and randomization to make up for discontinued subjects is not planned. This document clarifies a typographical error in the study protocol that incorrectly indicated a treatment difference of 2.5 points instead of 2 points, is of interest.

1.6. Randomization and Blinding

Double-Blind 24-Week Treatment Period (Part A):

Subjects who satisfy all the inclusion and none of the exclusion criteria will be randomly assigned in a 2:1 ratio to receive one of the following two treatment arms during the 24-week treatment phase according to a study randomization scheme:

- Arm 1: PTC857 (250 mg) administered orally BID for 24 weeks.
- Arm 2: Matching placebo administered orally BID for 24 weeks.

Investigators and site staff will be blinded to treatment assignments during the entire study period. Biostatistics staff who are directly involved in the analysis of the study results and members of the clinical study team will remain blinded to the treatment assignment throughout the conduct of Part A and will be unblinded after database lock for Part A.

2. ANALYSIS SETS

2.1. All Enrolled Set

The All-Enrolled set will contain all subjects who signed an informed consent for this study. This analysis set will be used to summarize subject disposition.

2.2. Intent-to-Treat (ITT) Analysis Sets

Intent-to-Treat 1 Analysis Set (ITT1)

All subjects who are randomized after the Screening Period and take at least 1 dose of double-blind study drug in the Treatment Period and who have a decrease in the ALSFRS-R score of ≤4 points during the Screening Period will be included in the ITT1 Analysis Set. The ITT1 Analysis Set will be used for analysis of the primary endpoint. Subjects will be analyzed according to their randomized treatment.

Intent-to-Treat 2 Analysis Set (ITT2)

All subjects who are randomized after the Screening Period and take at least 1 dose of double-blind study drug in the Treatment Period will be included in the ITT2 Analysis Set. Subjects will be analyzed according to their randomized treatment assignment. ITT2 analysis set will be used as supportive analysis population.

2.3. Safety Set

All subjects in the ITT2 Analysis Set who receive at least 1 dose of study drug will be included in the Safety Analysis Set. Subjects will be analyzed according to the actual treatment received.

2.4. Per Protocol Set

All subjects in the ITT1 Analysis Set who have no major protocol deviations that affect the validity of the efficacy measurements will be included in the PP Analysis Set. The PP Analysis Set will be used for sensitivity analysis of the efficacy endpoint. Classification of whether protocol deviations require exclusion from the ITT1 set will be assessed at a Blinded Data Review Meeting (BDRM) prior to database lock. Subjects who meet the following criteria will be excluded from the ITT1 population hereby forming the per-protocol analysis set:

(1) Received a protocol prohibited concomitant medication during the double-blind period.
   a. Received edaravone and sodium phenylbutyrate/taurursodiol in combination,
   b. Received a non-approved form of sodium phenylbutyrate/taurursodiol,
   c. Initiated a new approved ALS therapy during the double-blind period.

(2) Received study treatment different from the randomized treatment throughout the double-blind period

(3) Significant non-compliance to study drug

(4) Had significant inclusion or exclusion criteria violations

(5) Had protocol deviations which may confound results of study treatment effectiveness

(6) ALSFRS-R assessment on day -56, day 1 or day 169 performed by a non-certified rater.

This population will be to analyze the primary efficacy endpoint as supportive efficacy analysis. A separate document listing subjects excluded from the ITT1 population and reasons for exclusion will be finalized prior to treatment unblinding.

2.5. Pharmacokinetic Analysis Set

All subjects in the ITT2 Analysis Set who have at least 1 measurable post-baseline plasma or CSF PTC857 concentration will be included in the PK Analysis Set. Subjects with protocol violations that could affect the PK parameters will be assessed by the pharmacokineticist for inclusion into the PK analysis set. The pharmacokineticist may also exclude subjects or specific concentrations from the PK parameter calculation if required, for example if doses were missed or if samples were collected out of the allowed assessment windows. PK summaries and analyses will be conducted using the PK analysis set. The PK analysis set will be based on the actual treatment received.

2.6. Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the subject, investigator, or site staff. Examples of deviations include, but are not limited to, the following:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc. (either the tests were not done, the incorrect tests were done, or the tests were not done within the time frame specified in the protocol)
- Procedural deviations such as incorrect storage of study drug, failure to update the ICF when new risks become known, or failure to obtain IRB/IEC approvals for the protocol and ICF revisions

Major protocol deviations are defined in the study's Protocol Deviation Handling Plan and include quality issues that have a high likelihood to significantly impact a subject's rights, safety or well-being, or have a potentially significant impact on data integrity.

Protocol deviations will be documented separately in a stand-alone file before database lock which include deviation category (e.g., violation of inclusion and exclusion criteria at screening, use of excluded concomitant medications, received the wrong treatment or incorrect dose), deviation description, severity (minor/major), visit/time point for each deviation. .

Protocol deviations pertaining to the effects of the COVID-19 pandemic and related measures may be seen on this trial, and there may be an increase in the number of deviations seen due to the pandemic. All discussions pertaining to the COVID-19 pandemic and related protocol

deviations affecting inclusion/exclusion of analysis sets, and any adjustments to data handling and/or analysis of data will be documented in the blinded data review meeting (BDRM) Report. Both the BDRM Report and this document (SAP) will be finalized before database lock. A by-subject listing of major and minor protocol deviations will be provided for the safety analysis set.

3. GENERAL CONSIDERATIONS

3.1. General Considerations

Continuous data will be summarized using number of observations (n), arithmetic mean (mean), standard deviation (SD), median, minimum value (min) and maximum value (max) by treatment group. Additionally, for biomarker data, the summaries will include geometric means and standard deviations, as appropriate. Categorical variables will be summarized using the frequency count (n) and percentage (%) by treatment group. An overall/total group (i.e., sum of all treatment groups) will generally be presented additionally for summaries of disposition, demography, and baseline characteristics.

For all percentage calculations, the denominator will be the number of subjects in the analysis set for the treatment group, unless otherwise stated.

Only data from protocol scheduled visits will be included in the summary tables. Data from unscheduled visits will not be included in the summary tables but will be included in the by-subject listings. Descriptive summaries by visit and treatment will be provided for all the primary and secondary efficacy endpoints.

3.2. Data Definitions and Analysis Issues

3.2.1. Study/Onset Day

The study/onset day will be defined as the number of days since the first dose of study drug, which is assigned as Day 1 for analysis purposes. Study/onset day is calculated using the formula below:

Assessment Date Relative to First Dose of Study Drug	Study Day Calculation
Assessment/Event date prior to date of first dose	Date of assessment – Date of first dose of study drug
Assessment/Event date on or after Date of first dose	(Date of assessment – Date of first dose of study drug) +1

3.2.2. Baseline Definition and Change from Baseline

The Baseline Visit will be defined as Day 1 of the study. Unless otherwise stated, for each subject, baseline value of a parameter is defined as the last non-missing measurement taken at most one week prior to the first dose of study drug (including unscheduled assessments). If the last non-missing measurement and the date of first dose of study drug coincide, that measurement will be considered baseline, but AEs and medications commencing on the date of first dose of study drug will be considered post-baseline.

For the purposes of calculating summary statistics of the outcome measures, change from baseline will be derived as follows:

- Change from baseline = Post-baseline value – Baseline value

3.2.3. Duration of Event

Where the duration of an event is to be calculated, it will be derived as:

Duration (days) = (Event stop/end date – Event start date) + 1.

Duration (months) = Duration (days) / 30.4375

3.2.4. Retest and Unscheduled Visits

Unscheduled measurements and retests will not be included in by-visit summaries but will contribute to incidence of significant abnormality tables, where applicable.

In the case of a retest (same visit number assigned), the last available measurement for that visit will be used for by-visit summaries. Data listings will include all assessments, both scheduled and unscheduled.

3.2.5. Treatment Periods

Period	Definition
Pre-treatment	Date of Screening through one day prior Date of first dose
Double-blind Treatment Period	Date of first dose through to Date of last dose during Part A or Date of first dose through to one day prior first dose during open-label treatment period

3.2.6. Coding Dictionary

Where applicable, safety data will be coded using the following coding dictionaries:

Dictionary	Version
Medical Dictionary for Regulatory Activities (MedDRA)	25 or higher
World Health Organization Drug Dictionary (WHODrug Global)	WHODrug-Global-B3 Sep 2021 or later

3.2.7. ALS Functional Rating Scale - Revised (ALSFRS-R) score

The ALSFRS-R is a quickly administered (5-minute) ordinal rating scale that assesses the subjects' capability and independence in 12 functional activities across 4 subdomains of bodily function (bulbar, gross motor, fine motor, and respiratory) relevant in ALS. Each activity is recorded to the closest approximation from a list of 5 choices, scored 0 (total loss of function) to 4 (no loss of function), with the total score ranging from 0 to 48 and higher scores indicating less functional impairment (Cedarbaum 1999).

The score change from baseline will be calculated as the post baseline score minus the baseline score, so that a positive score for change from baseline would indicate improvement in function relative to baseline, while a negative score indicates worsened disease relative to baseline.

Subdomains	Items
Bulbar	Speech, Salivation and Swallowing
Fine Motor	Handwriting, Cutting Food/Handling Utensils and Dressing Hygiene
Gross Motor	Turning in Bed, Walking and Climbing Stairs
Respiratory	Dyspnea, Orthopnea and Respiratory Insufficiency
Scores for each item range from 0 to 4 (0 representing the worst/lowest functional rating and 4 the best rating)	

3.2.8. Pulmonary Function Tests

Pulmonary function tests are used to assess respiratory function. Pulmonary function tests (PFTs) will assess SVC and SNIP. Forced vital capacity, vital capacity, forced expiratory volume, and maximum inspiratory pressure will not be measured. Oxygen saturation (SpO_2), respiratory rate, and heart rate will be assessed in both upright and supine positions. The SNIP test will assess nasal pressure in each unilaterally occluded nostril during a maximal sniff to assess inspiratory muscle strength.

3.2.9. Modified Norris Scale

Modified Norris scale is used to assess the motor/limb and bulbar function. The Modified Norris Scale is a rating scale for ALS that consists of 2 parts: the Limb Norris Scale and the Norris Bulbar Scale. The Limb Norris Scale has 21 items to evaluate extremity function, and the Norris Bulbar Scale has 13 items to evaluate bulbar function. Each item is rated in 4 ordinal categories. The total score is calculated by summing all the scales (Norris 1974).

3.2.10. ALS Cognitive Behavioral Screen

The ALS CBS is a brief measure of cognition and behavior in patients with ALS. The ALS CBS is composed of 2 sections: cognitive and behavioral. The cognitive section includes commonly used elements of standard testing batteries, consisting of 8 tasks that assess attention, concentration, tracking/monitoring, and initiation and retrieval. Each subtest score ranges from 0–5 and the total score ranges from 0–20. The behavioral section is composed of questions sensitive to organic brain changes. It consists of fifteen 3-point Likert items (total score ranges from 0–45) questioning caregivers on patients' behavioral changes (3 = "no change"; 0 = "large change") and 4 "yes/no" questions on anxiety and depression.

3.2.11. ALSAQ-40 Assessment

The ALSAQ-40 is a disease-specific measure of health-related quality of life for ALS (Jenkinson 1999). It is specifically used to measure the subjective well-being of patients with ALS and provides scores for 5 scales: physical mobility (10 items: 1-10), activities of daily living and independence (10 items: 11-20), eating and drinking (3 items: 21-23), communication (7 items: 24-30) and emotional reactions (10 items: 31-40). If one of the question items of ALSAQ-40 is missing, the total score will be considered as a missing value. However, it may be included in the summary of the specific scales. Lower total scores for the ALSAQ-40 indicate better quality of life, with higher scores indicating poorer quality of life. Analyses will be performed on the total score.

3.2.12. EuroQol EQ-5D-5L

The EQ-5D-5L is a patient reported outcome measure (PROM) that determines a subject's health-related quality of life (www.euroqol.org). EQ-5D-5L consists of 2 components: The health state descriptive system and the visual analogue scale (EQ-VAS).

The health state descriptive system is a 5 Dimension (5-D) questionnaire covering 5 health related aspects: Mobility, self-care, usual activities, pain/discomfort and anxiety/depression. For each Dimension, the subject responds by selecting one of 5-levels (5L): 1=No problems; 2=Slight Problems; 3=Moderate Problems; 4=Severe Problems and 5=Extreme Problems.

The visual analogue scale (EQ-VAS) is a 20 cm vertical graduated visual analogue scale with numbers ranging from 0 (=worst imaginable health) to 100 (=best imaginable health). Subjects rate their overall health by placing an X mark on the scale to indicate their perceived overall health.

3.2.13. Survival

The survival endpoint is defined as the time from the randomization date to the date of death from any cause.

3.2.14. Combined Assessment of Function and Survival (CAFS)

CAFS is a joint rank test developed for ALS that evaluates function while appropriately accounting for missing data due to deaths in ALS (Berry 2013). CAFS ranks each subject according to their outcome, with the worst subject's clinical outcomes based on the survival time and the ALS Functional Rating Scale–Revised (ALSFRS-R) score at the timepoint of interest. Each subject's outcome is compared to every other subject's outcome, assigned a score, and the total scores are ranked. The mean rank score for each treatment group can then be calculated. A higher mean CAFS score indicates a better group outcome.

CAFS scoring: Subject's CAFS scores will be computed using the approach proposed by Berry (2013). Each subject is compared individually to all other subjects in the study. For each pairwise comparison of subjects, the participant who fares better earns a point (+1), and the one who fares worse loses a point (-1). In the case of a tie, no points are added or subtracted (0). The total score for each subject is the sum of the comparisons (+1, 0, -1) against all other subjects. The pairwise comparisons will be performed as follows: If both subjects die, the one surviving longer fared better; if only one survives then that subject fared better; and if both subjects survive, the one with the smaller decline (from baseline to 24 weeks) in ALSFRS-R fared better. If subjects discontinue prior to their week 24 assessment, comparison will be based on their imputed week-24 ALSFRS-R result (See Section 5.5.1.2 for multiple imputation details).

CAFS ranking: The ranking has the following characteristics: 1) the first subject who dies will have the lowest score and is ranked the lowest; 2) the last to die is ranked above all others who die; 3) among survivors, the subject with the largest decline in ALSFRS-R score at week 24 is ranked just above the last subject who died; 4) the surviving subject with the smallest decline from baseline in ALSFRS-R score at week 24 is ranked highest. An average rank score can then be calculated for each treatment group. A higher mean rank score indicates that subjects in that treatment group, on average, fared better.

3.3. Estimands

3.3.1. Estimand for Primary Efficacy (Combined Assessment of Function and Survival)

Research Question: What is the improvement in combined survival and activities of daily function between subjects treated with PTC857 versus Placebo after 24-weeks, in the population of patients with ALS as defined by the study's inclusion exclusion criteria, regardless of whether subjects complete their assigned treatment during the 24-week treatment period, or underwent a tracheostomy, or had permanent assisted ventilation?

Patient Population: All subjects who are randomized to a treatment assignment and take at least 1 dose of study drug during the double-blind treatment period, and who have a decrease in the ALSFRS-R score of ≤ 4 points during the Screening Period (ITT1 Population).

Endpoint: CAFS rank at Week 24

Population-level summary: Difference in the mean CAFS rank at Week 24 between PTC857 and Placebo.

Treatment Condition: PTC857 compared to Placebo, in the setting where patients have the option (at screening), to take SOC treatments for ALS that are approved in their country; patients are allowed to take either edaravone or sodium phenylbutyrate/taurursodiol as monotherapy or in combination with riluzole but not in combination with each other.

Intercurrent Event Strategy:

1. The intercurrent event of death will be accounted for as part of the composite CAFS score.
2. Treatment Discontinuation: Data collected after treatment discontinuation will be included and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy).
3. Initiation of new therapy for ALS: If subjects initiate new additional therapy for ALS after randomization their data will be analyzed according to the treatment group to which the participant was randomized, i.e., regardless of the initiation of new therapy for ALS. (Treatment Policy).
4. Tracheostomy: Data collected after a Tracheostomy will be included and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy).
5. Permanent Assisted Ventilation (PAV): Data collected after a PAV will be included and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy).

The same estimand framework (above) will be used to analyze the primary endpoint for the ITT2 population.

3.3.2. Estimands for Change from Baseline in ALSFRS-R at Week 24

Research Question: What is the difference in mean ALSFRS-R change from baseline between subjects treated with PTC857 versus Placebo after 24 weeks of treatment, in the population of patients with ALS as defined by the study's inclusion exclusion criteria, regardless of whether subjects complete their assigned treatment during the 24-week treatment period, or underwent a tracheostomy, or had permanent assisted ventilation?

Patient Population: All subjects who are randomized to a treatment assignment, take at least 1 dose of study drug during the double-blind treatment period, and who have a decrease in the ALSFRS-R score of ≤ 4 points during the Screening Period (ITT1 Population).

Endpoint: Change from baseline in ALS Functional Rating Scale-Revised (ALSFRS-R) at Week 24

Population-level summary: Difference in the mean ALSFRS-R change from baseline to Week 24 between PTC857 and Placebo.

Treatment Condition: PTC857 compared to Placebo, in the setting where patients have the option (at screening), to take SoC treatments for ALS that are approved in their country; patients are allowed to take either edaravone or sodium phenylbutyrate/taurursodiol as monotherapy or in combination with riluzole but not in combination with each other.

Intercurrent Event Strategy:

1. Death: Missing data after death up to week 24 will be implicitly imputed by means of mixed model repeated measures analysis (MMRM) assuming MAR. Analysis will be carried out on all available data. (Hypothetical)
2. Treatment Discontinuation: Data collected after treatment discontinuation will be included and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy)
3. Initiation of new therapy for ALS: If subjects initiate new additional therapy for ALS after randomization (for example a subject start taking edaravone at week 18 with or without discontinuing their baseline therapy), data collected from this point forward will be included and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy)
4. Tracheostomy: Data collected after a Tracheostomy will be included and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy)
5. Permanent Assisted Ventilation (PAV): Data collected after a PAV will be included and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy)

Other continuous endpoints will use a similar framework for the main estimand, with analysis via an MMRM model. This includes the Modified Norris Scale, ALS CBS, NfL and ALSAQ-40. This estimand framework will also be applied to the ITT2 population.

3.3.3. Estimands for Survival Time

The main estimand for survival time is as follows:

Research Question: How does survival time compare between PTC857 treatment versus Placebo groups, in the population of patients with ALS as defined by the study's inclusion exclusion criteria?

Patient Population: All subjects who are randomized to a treatment assignment after the Screening Period, take at least 1 dose of study drug during the double-blind treatment period, and who have a decrease in the ALSFRS-R score of ≤ 4 points during the Screening Period (ITT1 Population).

Endpoint: Time to death

Population-level summary: The hazard ratio between PTC857 and Placebo for the event of death.

Treatment Condition: PTC857 compared to Placebo, in the setting where patients have the option (at screening), to take SOC treatments for ALS that are approved in their country; patients are

allowed to take either edaravone or sodium phenylbutyrate/taurursodiol as monotherapy or in combination with riluzole but not in combination with each other.

Intercurrent Event Strategy:

1. Treatment Discontinuation: Survival data after a treatment discontinuation will be included up through the date of death or last follow-up date and analyzed according to the treatment group to which the participant was randomized (Treatment Policy).
2. Initiation of new therapy for ALS: If subjects initiate new additional therapy for ALS after randomization (for example a subject start taking edaravone at week18 without discontinuing their baseline therapy), survival data after this event will be included up through the date of death or last follow-up date and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy).
3. Tracheostomy: Survival data after a Tracheostomy will be included up through the date of death or last follow-up date and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy).
4. Permanent Assisted Ventilation (PAV): Survival data after a PAV will be included up through the date of death or last follow-up date and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy).

This estimand framework will also be applied to the ITT2 population.

3.3.3.1. Respiratory-Support free survival time - A supportive estimand for the survival time

Research Question: How does respiratory-support free survival time compare between PTC857 treatment versus Placebo groups, in the population of patients with ALS as defined by the study's inclusion exclusion criteria?

Patient Population: All subjects who are randomized to a treatment assignment after the Screening Period, take at least 1 dose of study drug during the double-blind treatment period, and who have a decrease in the ALSFRS-R score of ≤ 4 points during the Screening Period (ITT1 Population).

Endpoint: Time to death or needing respiratory support (Tracheostomy and/or Permanent Assisted Ventilation [PAV])

Population-level summary: The hazard ratio between PTC857 and Placebo for the event of death or needing respiratory support/intubation.

Treatment Condition: PTC857 compared to Placebo, in the setting where patients have the option (at screening), to take SOC treatments for ALS that are approved in their country; patients are allowed to take either edaravone or sodium phenylbutyrate/taurursodiol as monotherapy or in combination with riluzole but not in combination with each other.

Intercurrent Event Strategy:

1. Treatment Discontinuation: Survival data after a treatment discontinuation will be included up through the date of event (death/respiratory support) or last follow-up date and analyzed according to the treatment group to which the participant was randomized (Treatment Policy).

2. Initiation of new therapy for ALS: If subjects initiate new additional therapy for ALS after randomization (for example a subject start taking edaravone at week18 without discontinuing their baseline therapy), survival data after this event will be included up through the date of event (death/respiratory support) or last follow-up date and analyzed according to the treatment group to which the participant was randomized. (Treatment Policy).
3. Tracheostomy: The intercurrent event of tracheostomy will be accounted for as part of the composite endpoint (death or needing respiratory support/intubation) (Composite).
4. Permanent Assisted Ventilation (PAV): The intercurrent event of PAV will be accounted for as part of the composite endpoint (death or needing respiratory support/intubation). (Composite).

This estimand framework will also be applied to the ITT2 population.

3.4. Interim Analysis

No formal interim analyses are planned for this study.

The analysis of the data for the double-blind treatment period (Part A) as outlined in this SAP will be produced after the lock of database for Part A.

3.5. Changes to Protocol Specified Analysis

As described in Section 1.1, protocol amendment 4.0 allowed for randomization of subjects receiving edaravone or sodium phenylbutyrate/taurursodiol either in combination with riluzole or as monotherapy. It was anticipated that a significant number of subjects would opt for treatment with these therapies. As the study progressed and randomization continued, it became evident that the adoption rate of these treatments was markedly lower than anticipated. A vast majority of enrolled patients were only on riluzole as monotherapy with a small minority on either edaravone or sodium P/T as monotherapy or in combination with riluzole. To mitigate potential issues with statistical modeling arising from sparse strata, the protocol specified analyses described in Section 5, will use a binary form of the ALS treatment stratification factor, defined as the use of approved background ALS therapy at screening (Yes or No), rather than the original three-level stratification variable employed during randomization. Should modeling challenges due to sparse strata persist, a partially stratified analysis (excluding either one of the two stratification factors) or a fully unstratified analysis (exclusion of both factors) will be considered.

Derivation of the binary stratification variable (use of approved background ALS therapy at screening) will be based on the stratum subjects were assigned to during randomization irrespective of stratification errors made by the sites. An example of such a stratification error is a subject on stable edaravone treatment being randomized out of the sodium phenylbutyrate/taurursodiol stratum.

A list of all stratification errors including those related to the other stratification variable (decline in ALSFRS-R during the screening period), will be provided.

4. SUBJECT DATA

4.1. Subject Disposition

Subject disposition data will be listed and summarized. A summary table of subject disposition will be produced reflecting:

- Screened (or enrolled) subjects.
- Number of subjects in the ITT1, ITT2, Safety, Per-protocol and Pharmacokinetic analysis sets
- Subjects who complete the study treatment
- Subjects who early terminate the study treatment and reasons for early termination
- Subjects who early terminate the study treatment but continue onto the survival follow-up
- Subjects who early terminate the study and reasons for early termination

Screen Failure subjects will be listed.

For subjects who fail screening, the reasons for screen failure including details on which inclusion / exclusion criteria were not met will be listed.

For the enrolled population, the number and percentage of participants in each analysis population will be presented.

4.2. Demographics and Baseline Characteristics

Subjects' demographics and baseline characteristics will be summarized overall and by treatment group for the ITT1, ITT2, and Safety analysis sets.

Continuous variables (e.g., age and weight) will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). Qualitative variables (e.g., sex, race, and ethnicity) will be summarized with counts and percentages.

The demographic parameters will include age category (<65 and ≥65 years), gender, race, and ethnicity. Age will also be summarized as a continuous variable. The baseline parameters include, height, body weight, BMI, ALSFRS-R score, amount of change in ALSFRS-R score during the screening period (<1, 1-2, 3-4, or >4 points total loss) use of standard of care (SoC) medications for ALS at baseline, and disease characteristics at screening.

By-subject listings of demographic and other baseline characteristics will be provided for all randomized subjects. These listings will use flag variables to identify subjects in various analysis sets (ITT1, ITT2, safety and per-protocol).

4.3. Disease Characteristics

Subjects' disease characteristics will be summarized overall and by treatment group for the ITT1, ITT2, and Safety analysis sets. By-subject listings of disease characteristics will also be provided. Duration of symptoms (months) and months since diagnosis will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). The following disease characteristics will be summarized with counts and percentages (n (%)):

- Site of symptom onset
- Family history
- El Escorial criteria at screening
- Pre-ALS impairment
- Months since ALS symptom onset
- Months since diagnosis
- Site of onset
- Diagnosis delay
- Derived King's Clinical Stage (Balendra 2014 also see Appendix 5)
- TRICALS Risk Profile

The TRICALS risk profile (van Eijk 2021) is based on the European-Network-to-Cure-ALS survival prediction model, a cross-validated model to predict the composite endpoint death or respiratory insufficiency based on data of 11,475 patients with ALS (Westeneng 2018). The TRICALS Risk Profile is a weighted average of seven pre-randomization patient characteristics, including: Percent predicted vital capacity, age at onset in years, diagnostic delay (months from symptom onset to diagnosis), presence of frontotemporal dementia, progression rate of the ALS functional rating scale (points per month), Bulbar onset of ALS, and an El Escorial criteria of clinical definite ALS at screening. The range of possible values for this summary predictor is from approximately −12.0 to 0.0 with higher scores indicating a poorer prognosis. Section 13 (Appendix 3) provides details on its derivation.

4.4. Medical History

Medical history will be coded using a central coding dictionary, the Medical Dictionary for Regulatory Activities (MedDRA) terminology.

Medical history will be defined as any significant past medical conditions that ended before screening or current medical conditions that were ongoing at Screening. Medical history will be summarized separately by system organ class (SOC) and preferred term (PT) overall and for each treatment group for the safety analysis set. Summaries will be sorted alphabetically by SOC, and by descending order of frequency for PTs according to the total column. Subjects with more than one of the same PTs within an SOC will be counted only once. A summary of medical history by randomized treatment arm will be provided for the ITT1 and Safety analyses sets. In addition, a by-subject listing will also be provided for the safety analysis set.

4.5. Prior Medications/Procedures and Concomitant Medications/Procedures

Prior medications are medications which started and stopped before the first dose of study drug. Any changes to prior medications (dose, regimen, etc.) during the study will be considered a new concomitant medication.

Concomitant medications/procedure are medications/procedures which started prior to or after the first dose of study drug and ended after the date of first dose of study drug or ongoing at the

end of the study. If a medication or procedure cannot be classified as "prior" or "concomitant" after applying imputation rules for missing/incomplete dates, it will be classified as concomitant.

Prior and concomitant medications will be coded using the latest available version of the Anatomical Therapeutic Chemical (ATC) classification text from the World Health Organization Drug Dictionary (WHO DD) and summarized separately. The number and percentage of subjects taking a medication will be displayed by therapeutic class (ATC Level 3) and preferred name and treatment group. Summaries will be sorted alphabetically by ATC-Level 3, and by descending order of frequency for preferred name according to the total column. Subjects with more than one of the same preferred names within an ATC-Level 3 will be counted only once.

Prior and concomitant procedures will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology.

A by-subject listing of prior and concomitant medications will be provided for the safety analysis set.

The count and percentage of subjects who have received prior and concomitant procedures will be summarized by treatment group, in addition, by-subject listing of prior and concomitant procedures will be provided for the safety analysis set.

4.6. Multiplicity

A fixed sequence testing procedure will be employed for selected efficacy endpoints. If the primary endpoint is statistically significant at a 2-sided 0.05 level, the secondary endpoints will then be tested sequentially in the order listed below, each at a 2-sided 0.05 alpha level. Testing within the specified sequence will stop if a null hypothesis in the sequence fails to be rejected.

(1) Change from baseline in ALSFRS-R at Week 24 in the ITT1 analysis population

(2) Change from baseline in ALSFRS-R at Week 24 in the ITT2 analysis population

(3) Subject ranks based on the CAFS at Week 24 in the ITT2 analysis population

(4) Change from baseline in total score of the Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) after 24 weeks of treatment, in the ITT1 analysis population

(5) Change from baseline in log transformed plasma NfL, in the ITT1 analysis population

There will be no interim analysis.

4.7. Missing Data

For the analysis of CAFS, missing ALFSRS-R data will be assumed to be missing at random (MAR) and will be imputed using multiple imputation.

4.8. Treatment Compliance

Percent compliance will be calculated as the amount of dose taken divided by the amount of dose that should have been taken multiplied by 100. The following formula will be used to calculate compliance.

$$Compliance\ (\%) = \frac{100 \times (2 \times treatment\ duration\ in\ days - number\ of\ missed\ doses)}{2 \times treatment\ duration\ in\ days}$$

The extent of exposure to study medication (treatment duration) in days will be calculated as the last dose date minus the first dose date + 1.

Exposure (number of days) and compliance will be summarized descriptively (number of doses taken / number of doses expected and percentage) for the ITT1, ITT2 and safety analysis sets.

4.9. Visit Windows for The Early Termination Visit and Unscheduled Visits

If a subject completely withdraws from the study prematurely, and unless consent is withdrawn, all efforts are made to collect efficacy and safety data and to complete the Early Termination Visit (ET).

Follow-up efficacy data collected at the early termination visit will be incorporated into the study visit schedule using the visit windows, defined in Table **1** and Table **2**. Similarly, follow-up efficacy data collected during unscheduled visits or make-up visits will also use the same visit windows. If a follow-up visit (ET) or unscheduled visit falls in the same visit window as a scheduled visit, then the scheduled visit data will be used. If an unscheduled visit and ET visit fall within the same window, then the visit closest to the target day will be used.

Table 1: Visit Windows for Early Termination and Unscheduled Visits

	ALSFRS-R		
Visit	Lower Bound	Target Day	Upper Bound
Day 29	15	29	42
Day 57	43	57	70
Day 85	71	85	98
Day 113	99	113	126
Day 141	127	141	154
Day 169	155	169	182

Table 2: Visit Windows for Early Termination and Unscheduled Visits

	SVC, ALSAQ-40, Norris Scale, and plasma NfL		
Visit	Lower Bound	Target Day	Upper Bound
Day 85	64	85	106
Day 169	148	169	190

5. EFFICACY ANALYSIS

5.1. Analysis of the Primary Efficacy Endpoint

The primary efficacy variable, subject ranks based on the combined assessment of function and survival (CAFS) after 24 weeks of treatment will be performed using an analysis of covariance model (ANCOVA) following multiple imputation. The estimand framework described in Section 3.3.1 will be used. The ANCOVA model will contain the following main effects: treatment (PTC857 or placebo), the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2, or 3-4-points total loss), TRICALS risk profile (continuous), use of Approved Background Therapy for ALS (Yes [either riluzole, edaravone, or sodium phenylbutyrate/taurursodiol], No). Baseline ALSFRS-R score (continuous) and The TRICALS risk profile (continuous) will be included as covariates.

Subjects who discontinue from the study prior to week 24 will have their week 24 ALSFRS-R score imputed using the same datasets described in Section 5.5.1.2. In each of the 100 imputed datasets, subjects who die will have their death day (study day) incorporated as part of the combined assessment of function and survival.

The p-value for the treatment comparison (PTC857 versus Placebo) at week 24, estimate of LSMEANS for the treatment differences (PTC857 – Placebo), and the two-sided 95% confidence interval of the LSMEANS difference will be presented for the ITT1 analysis set (obtained from PROC MIANALYZE in SAS). If the p-value for the treatment arm comparisons at week 24 is less than 0.05, then the study will be declared as positive.

This analysis will also be repeated for the ITT2 analysis set and a nominal p-value for treatment arm comparison at week 24 presented.

In addition, averaged win probability (Rubin's rule) and a win-odds, will also be presented.

$$Win\ probability(\theta) = 0.5 + \frac{\bar{R}_i - \bar{R}_j}{N_i + N_j}$$

Where:
$\bar{R}_{\cdot} = mean\ ranks\ for\ treatment\ groups_{i,j}$
$N_{\cdot} = No.\ subjects\ in\ treatment\ groups_{i,j}$

$$Win\ odds = \frac{\theta}{(1 - \theta)}$$

Given the difficulties in interpreting and quantifying the treatment effect from the CAFS analysis, results from the MMRM analysis in ALSFRS at week 24 (Section 5.5) will be used to describe the treatment effect in terms of loss of function). Similarly, survival differences will be described using results from the Survival analysis (Section 5.7)

5.2. Sensitivity Analysis – Unstratified Analysis

The primary efficacy analysis described in Section 5.1 will be repeated without including the two stratification factors (change in ALSFRS-R score during the 8-week Screening Period and standard of care therapy for ALS).

5.3. Sensitivity Analysis – Actual Stratification

A sensitivity analysis to determine the effect of any incorrect stratification during randomization on the primary analysis will also be performed. The primary analysis (Section 5.1) will be

repeated using the actual stratification, defined as the stratification level they should have been randomized from.

5.4. Per protocol Analysis Set

The primary efficacy analysis described in Section 5.1 will be repeated using the Per protocol analysis set described in Section 2.4. For this analysis the seed to be used in the MI model is 587432

5.5. Analysis of Secondary Efficacy Endpoints

For all efficacy analyses described in this Section and in Sections 5.6 through 5.8, partially stratified analysis (excluding either one of the two stratification factors) or fully unstratified analyses (exclusion of both factors) may be considered should there be issues with statistical modeling arising from sparse strata.

5.5.1. Change from Baseline in ALSFRS-R

5.5.1.1. Mixed Model Repeated Measures (MMRM)

Change from baseline in ALSFRS-R at Week 24 will be evaluated by means of a mixed model repeated measures analysis using all available ALSFRS-R scores up to Week 24. The estimand framework described in Section 3.3.2 will be used. The model will include fixed categorical effects of treatment (PTC857 or placebo), use of approved background therapy for ALS (Yes vs No), the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2 or 3-4 points total loss), time of collection (nominal week at the visit as a categorical variable), as well as the continuous, fixed covariates of baseline ALSFRS-R, and The TRICALS risk profile. In addition, the following interaction terms will be included: Time by treatment, time by baseline ALSFRS-R, and TRICALS risk profile by time. Subject will be included as a random effect. The Restricted Maximum Likelihood (REML) estimation approach will be used with the variance-covariance structure set as unstructured (UN). Other types of covariance matrices will be explored in the case of non-convergence of the UN. Subjects with no post baseline results for the outcome variable will be excluded. This analysis will be performed for both the ITT1 and ITT2 analysis sets.

Observed and change-from-baseline values will also be summarized descriptively at each visit.

In supplementary analyses, score changes from baseline at Week 24 for the ALSFRS-R subdomains will be analyzed under the same estimand framework where MMRM models will be used to implicitly impute missing data. Nominal P-values for the treatment arm comparison, estimate of LSMEANS for the treatment differences (PTC857 – Placebo) at Week 24, and the two-sided 95% confidence intervals of the LSMEANS difference will be presented.

5.5.1.2. Analysis of Covariance Model (ANCOVA) with Multiple Imputation:

An additional analysis to evaluate change from baseline in ALSFRS-R will be performed using an analysis of covariance model (ANCOVA) following multiple imputation (MAR assumption). Missing ALSFRS-R scores, (including those missing due to death) will be imputed using multiple imputation (MI), 100 imputations will be generated using PROC MI in SAS. The Fully Conditional Specification (FCS) model using the regression method will be used.

The ANCOVA model will contain the change from baseline in ALSFRS-R as the response variable and will include the following main effects: Treatment group (PTC857, or Placebo), the amount of change in the subject's ALSFRS-R score during the Screening Period (<1 point, 1-2-points or 3-4-points total loss), use of approved background therapy for ALS (Yes vs No). Baseline ALSFRS-R score (continuous) and The TRICALS Risk Profile (continuous) will be included as covariates.

The seed to be used in the MI model is 20221219.

For each imputed complete data set, the change from baseline in ALSFRS will be analyzed at week 24 using the ANCOVA analysis model.

The 100 estimates and standard errors will then be combined using Rubin's rule to produce a single estimate with standard error, confidence interval and nominal p-value using the PROC MIANALYZE procedure in SAS.

5.5.1.3. Pattern Mixture Model - Missing not at random assumption:

A sensitivity analysis for the primary efficacy endpoint, ALSFRS-R score change from baseline at Week 24, will handle missing efficacy data using the assumption that data are missing not at random using a control-based Pattern-Mixture Model (PMM). This method will assume that participants who discontinue from study treatment early will have an ALSFRS-R score that is similar to participants on placebo. For the PMM model, 100 imputations will be generated using PROC MI of SAS. The seed to be used for this analysis will be 1001.

5.5.2. Analysis of Other Continuous Secondary Endpoints

The following secondary (continuous) endpoints will be analyzed for both the ITT1 and ITT2 analysis sets under the estimand framework described in Section 3.3.2, where an MMRM is used to implicitly impute missing data. The MMRM models will include fixed categorical effects of treatment (PTC857 or placebo), use of approved background therapy for ALS (Yes vs No), the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2 or 3-4 points total loss), time of collection (nominal week at the visit as a categorical variable), as well as the continuous, fixed covariates of baseline endpoint variable (e.g., ALSFRS-R, ALSAQ-40), and The TRICALS risk profile. In addition, the following interaction terms will be included: Time by treatment, time by baseline value of endpoint, and TRICALS risk profile by time.

P-values for the treatment arm comparison at week 24, estimate of LSMEANS for the treatment differences (PTC857 – Placebo), and the two-sided 95% confidence intervals of the LSMEANS difference will be presented:

- Change from baseline in ALSFRS-R in the Intent-to-Treat 2 (ITT2) Analysis Set after 24 weeks of treatment.
- Quality of life as assessed by Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) after 24 weeks of treatment, for ITT1 and ITT2 analysis sets.
- Change from baseline in Modified Norris Scale total score, limbs score and bulbar score at Week 24, for ITT1 and ITT2 analysis sets.

- Change from baseline in slow vital capacity (SVC) as assessed by pulmonary function tests (PFTs) after 24 weeks of treatment for the ITT1 and ITT2 analysis sets.

To assess the change from baseline in plasma NfL after 24 weeks of treatment, log transformation of the plasma NfL results will be performed. A MMRM analysis will be performed, this analysis will include fixed categorical effects of treatment (PTC857 or placebo), use of approved background therapy for ALS (Yes vs No), time of collection (nominal week at the visit as a categorical variable), as well as the continuous, fixed covariates of baseline log plasma NfL, and The TRICALS risk profile. In addition, the following interaction terms will be included: Time by treatment, time by baseline log plasma NfL, and TRICALS risk profile by time. Geometric means and mean ratios to baseline, as well as the difference in geometric mean ratios for those in the PTC875 arm to those in the Placebo arm will be reported, together with 95% confidence intervals and p-values, for the ITT1 and ITT2 analysis sets. For this analysis values of plasma NfL that are BLQ will be set to half of LLOQ.

5.6. Subgroup Analyses for Efficacy

To explore the uniformity of the overall treatment effect in subgroups, change from baseline in ALSFRS-R at Week 24 for the subgroups listed below. This analysis will be performed using the MMRM analysis described in Section 5.2, with a treatment by subgroup interaction term added to the model. Subgroups with too few subjects resulting in model non-convergence will be summarized using descriptive summaries of the change from baseline in ALSFRS-R score at week 24.

- Sex (male, female)
- Race (white, non-white)
- Geographic region (North America, Europe, South America, Asia)
- Age at baseline (<65, ≥ 65 years)
- Slow vital capacity at baseline (<80%, ≥ 80%)
- Definite and probable ALS at screening
- Duration of symptoms (<12 months, ≥ 12 months)
- TRICALS Risk Profile (< -4, ≥ -4)
- Kings' Stage (1, 2, 3)
- Plasma NfL at baseline (< median value, ≥ median value)
- Decline in ALSFRS-R score during the 8-week screening period (1-2, 2-4, 1-4)
- Use of approved background therapy for ALS (Yes, No)
- Decline in ALSFRS-R score during the 8-week screening period (≤4 vs >4) [*]
- Site of ALS onset (limbs, bulbar, other)
- Baseline ALSFRS-R (<41, >=41)
- Family history of ALS (yes, no)

[*] ITT2 analysis set

5.7. Overall Survival

Overall survival (OS) is defined as the time in months from the date of first dose to the date of death from any cause or date last known alive for those who did not die.

Specifically,

OS = Date of death or date last known alive – Date of first dose + 1

Subjects will be censored at the last date they are known to be alive. The last known alive date will be the last date of any subject record in the study database. The date may be the last visit date or last contact date that the subject is known to be alive. Subjects who only have baseline record will be censored at the first dose date.

OS will be analyzed using KM methods. A summary table of the number of deaths, the number of censored subjects, and KM estimates (25th, 50th, 75th percentile of OS along with the 95% CI) will be presented. Kaplan-Meier survival curves will also be presented.

A stratified Cox Proportional Hazards regression model will be used to estimate the hazard ratio of PTC857 compared to Placebo. The model will be stratified by the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2 and 3-4-points total loss), and use of approved background therapy for ALS (Yes vs No). In addition, baseline ALSFRS-R score and the TRICALS risk profile and ALSFRS-R score at baseline will be included in the model as covariates. Ties in survival time will be handled using the Breslow method. Hazard ratio between the treatment groups and the corresponding 95% confidence intervals will be presented.

5.8. Exploratory Endpoints

Exploratory endpoints will be summarized for both the ITT1 and ITT2 analysis sets. Summary statistics by nominal timepoint will be provided for all the exploratory endpoints, including ALS-CBS, EQ5D-5L, and biomarkers (plasma hydroxyeicosatetraenoic acid [HETE], plasma ferritin, 4-hydroxynonenal [4HNE], urine p75 neurotrophin receptor [p75NTR], and cerebrospinal fluid NfL). Values of biomarkers and CSF NfL that are BLQ will be set to half of LLOQ.

ALS-CBS total score will be analyzed using the same methodology described for secondary (continuous) endpoints in Section 5.5.1.1. P-values for the treatment arm comparison at week 24, estimate of LSMEANS for the treatment differences (PTC857 – Placebo), and the two-sided 95% confidence intervals of the LSMEANS difference will be presented.

6. SAFETY ANALYSES

All analyses described in this section will be performed on the Safety Analysis set and will be presented by treatment group (actual treatment received PTC857 vs Placebo). The results will be descriptive in nature. There will be no statistical comparisons between the treatment groups for safety data, unless otherwise specified within the relevant section. All data will be summarized and listed.

Safety assessments will include adverse events, laboratory assessments, vital signs, 12-Lead electrocardiogram, physical examination, and Columbia-Suicide Severity Rating Scale (C-SSRS).

6.1. Extent of Exposure

The extent of exposure to study drug will be summarized descriptively. The summaries will include mean, SD, median, minimum, and maximum values.

The extent of study drug exposure for the double-blind treatment period (Part A) is defined as the difference between the dates of the last dose of study medication and the first dose study drug in the double-blind treatment period plus 1.

6.2. Adverse Events

Adverse events will be collected and recorded from the time subjects sign the informed consent form to the end of the follow-up period. All AEs will be classified by primary system organ class (SOC) and preferred term (PT) according to MedDRA coding dictionary. A treatment-emergent adverse event (TEAE) is an AE that begins after the first administration of study drug or any existing AEs that worsens after the first dose of study drug. All reported AEs will be listed, but only TEAEs will be summarized in tables.

AEs with an onset from day 1 of the double-blind treatment period up to and including 30 days after the last dose of study drug in the double-blind treatment period (or up to and not including the start date of the LTE period whichever comes first) will be considered as occurring during the double-blind treatment period.

An overall summary of TEAEs presenting number and percentage of subjects reporting at least one TEAE for the categories presented below:

- Subjects with at least one TEAE
- Subjects with at least one TEAE related to study drug
- Subjects with at least one serious TEAE
- Subjects with at least one non-serious TEAE
- Subjects with at least one serious TEAE related to study drug
- Subjects with at least one TEAE leading to premature discontinuation of study drug
- Subjects with at least one TEAE leading to death
- TEAEs by highest relationship to study drug
- TEAEs by highest severity

The following summaries will be tabulated:

- TEAEs overall and by SOC and PT
- TEAEs occurring in ≥ 5% of subjects within a treatment group, by PT
- TEAEs by PT
- Study drug related TEAEs, overall and by SOC and PT
- TEAEs by maximum severity, overall and by SOC and PT
- Grade 3 or Higher TEAEs by SOC and PT
- Grade 3 or higher related TEAE by SOC and PT
- TEAEs by highest relationship to study drug
- Serious TEAEs, overall and by SOC and PT
- Serious TEAEs, overall and by severity grade, SOC and PT
- Non-serious TEAEs, overall and by severity grade, SOC and PT
- TEAEs leading to discontinuation of study drug, overall and by SOC and PT
- TEAEs leading to death, overall and by SOC and PT

TEAEs with "possibly related", "probably related" or "related" to study drug based on the investigator assessment will be considered as drug related TEAEs. TEAEs with missing causality, if any, will be assumed as 'Related AEs'.

For summaries by SOC and PT, a subject will be counted only once at the SOC level and once at each PT within the SOC level, even if the subject experienced more than one AE within a specific SOC and PT. For summaries by SOC, PT, and maximum severity, a subject will be counted only once at the maximum severity level for which the event occurred at the SOC level and at the maximum severity level for which the event occurred for each unique PT within that SOC level. Therefore, subjects will contribute to only one severity level within a PT or SOC.

Summaries presenting frequency of TEAEs by SOC and PT will be ordered by overall descending frequency of SOC and then, within a SOC, by overall descending frequency of PT. Summaries presenting TEAEs by PT only will be ordered by overall descending frequency of PT.

Subject listings of all adverse events will be provided. Deaths and other serious or clinically significant non-fatal adverse events will be listed separately.

6.2.1. Exposure Adjusted incidence rates (EAIR)

Exposure-adjusted incidence rates (EAIRs) of treatment-emergent adverse events (TEAEs) will be presented by treatment group, MedDRA System Organ Class (SOC), and preferred term, as well as by treatment group and MedDRA preferred term. For each treatment group, the EAIR of a specific treatment-emergent event is defined as the number of subjects experiencing the event at least once, divided by the total exposure-adjusted follow-up time in person-years. EAIRs will be presented as incidence rates per person-years of follow-up.

For subjects who experience the event, the exposure-adjusted follow-up time will be calculated from the first exposure (to PTC857 or Placebo) until the first occurrence of the event. For subjects who do not experience the event (censored), the follow-up time will be calculated from the first dose until their last safety follow-up date while on Part A. This could either be the date of completion of Part A safety follow-up, the date of death if the subject died before experiencing the event, or the date of last on-treatment follow-up if the subject was lost to follow-up.

Missing or partial AE onset dates will be imputed using the imputation rules described in Section 12 (Appendix 2).

6.2.2. Adverse Events Indicative of ALS Disease Progression

TEAEs that are indicative of ALS disease progression will be tabulated by treatment arm. The following list of MedDRA preferred terms will be used to define TEAEs that are indicative of disease progression.

• Acute respiratory failure	• Increased bronchial secretion	• Salivary hypersecretion
• Affect lability	• Increased upper airway secretion	• Suicidal ideation
• Amyotrophic lateral sclerosis	• Muscle spasms	• Upper respiratory tract infection
• Asthenia	• Muscle spasticity	• Urge incontinence
• Balance disorder	• Muscular weakness	• Urinary retention
• Cardio-respiratory arrest	• Musculoskeletal stiffness	• Urinary tract infection **in males**
• Death	• Myalgia	• Urinary tract inflammation **in males**
• Dysgraphia	• Neurogenic bladder	• Viral upper respiratory tract infection
• Dysphagia	• Oral candidiasis	• Vocal cord paralysis
• Dyspnoea	• Oral fungal infection	• Weight decreased
• Fall	• Respiratory failure	
• Fatigue	• Respiratory tract congestion	
• Hypotonia	• Respiratory tract infection	
• Hypoxia	• Respiratory tract infection viral	

6.2.3. Disproportionate rate of Disease Progression by Treatment Received

To evaluate whether treatment with PTC857 may accelerate ALS disease progression, an analysis of participants with a disproportionate rate of disease progression by treatment assignment will be performed to further inform benefit-risk assessment. For this analysis the number and percent of subjects with declines in ALSFRS-R that are greater than or equal to 18 points at the 24-week visit will be tabulated by treatment arm.

6.3. Laboratory Values

All clinical laboratory analyses will be performed by the central laboratory. The clinical laboratory assessment of hematology, clinical chemistry, and urinalysis will be obtained at scheduled visits. The following parameters are collected during the double-blind period at the specified visits as per protocol:

Parameter(s)	Scheduled Visit(s)
Hematology: Hemoglobin, Hematocrit, RBC count, Reticulocytes, Platelet count, WBC count, Neutrophils, Eosinophils, Monocytes, Basophils, Lymphocytes, Mean corpuscular volume	Screening, Day 1, Day 29, Day 57, Day 85, Day 169, Day 197, ET
Clinical Chemistry: Urea, Creatinine, Fasting glucose, Sodium, Potassium, ALT, AST, GGT, Bilirubin (total, direct/indirect), Alkaline phosphatase, Albumin,	Screening, Day 1, Day 29, Day 57, Day 85, Day 169, Day 197ET
Lipid Profiles: Cholesterol (total), LDL, HDL, Triglycerides, LDH	Screening, Day 1, Day 14, Day 29, Day 57, Day 85, Day 169, Day 183, Day 197, and ET
Urinalysis: Urobilinogen, Nitrite, pH, Glucose, Total protein, Erythrocytes, Leukocytes, Ketones, Microscopy, Specific gravity, Bilirubin	Screening, Day 1, Day 85, Day 169, and ET
Coagulation: PT [sec], PT [INR], aPTT	Screening, Day 1, Day 29, Day 57, Day 85, Day 169, Day 197, and ET
Pregnancy Test: β-HCG, FSH	Screening, Day 1, Day 29, Day 57, Day 85, Day113, Day141, Day 169, Day 197, and ET
Note: Follow-up Visit on Day 197 is only for subjects who choose not to enter the LTE Period (Part B).	

Separate summary tables and listings will be produced for each of the separate groups above (e.g., separate table for hematology and clinical chemistry etc.); however, urinalysis, and serum and urine pregnancy tests will be listed only. The International System of units (SI units) will be used for all summary and data listing displays unless otherwise indicated.

Laboratory data will be summarized descriptively by visit for each laboratory parameter by treatment group and overall. Both observed results and changes from baseline will be summarized for continuous variables. Laboratory values will be categorized as clinical grade (low/normal/high) according to normal ranges. Shifts from baseline to the last on-treatment grade and the worst on-treatment grade will be presented. For these shift summaries, for the last on-treatment summary only scheduled visits will be taken; for the worst on-treatment summaries unscheduled assessments will be included in the assessment of worst. Both worst low and worst high will be summarized.

Laboratory values that are below the limit of quantification will be imputed as the lower-limit of quantification, similarly, values above the limit of quantification will be imputed as the upper-limit of quantification for the summary statistics. But for listings, the values will be presented as recorded.

By-subject listings of all the laboratory parameters will be presented that clearly indicate out of normal range values and possible abnormal values for the safety analysis set.

6.4. Vital Signs

Vital signs including systolic and diastolic blood pressure (mmHg), pulse rate (beats/min), body temperature ($^{\circ}$C), Oxygen saturation (SpO_2), and respiratory rate (breaths/min) will be evaluated

at Screening, Day 1, Day 29, Day 57, Day 85, Day 113, Day 141, and Day 169, as well as the ET visit where applicable.

For convenience, the conversion for temperature: Temperature (in °C) = (Temperature [in °F]-32) x (5/9).

Absolute results and changes from baseline will be summarized descriptively by visit for each parameter, by treatment group and overall.

6.5. 12-Lead Electrocardiogram

The following 12-lead ECG will be performed at Screening, Day 1, Day 85, and Day 169, as well as the ET visit where applicable.

- Heart rate (bpm)
- QRS Interval (msec)
- PR Interval (msec)
- QT interval (msec)
- QTcF Interval (msec)
- Overall Interpretation

Descriptive summaries will be presented for the ECG parameter values and change from baseline by visit for all quantitative measurements. Overall interpretation of ECG assessment will be summarized by counts and percentages. QT and QTcF will be categorized as follows, and the category based on actual value will be summarized by treatment group at each visit as well as the category at each post-baseline visit based on change from baseline per the following criteria:

Type of Value	Category 1	Category 2	Category 3
Actual value	> 450 ms	> 480 ms	>500 ms
Change from Baseline	> 30 ms	>60 ms	

A by-subject listing of all ECG parameters will be provided.

6.6. Physical Examination

A detailed physical examination will be performed at Screening visit and targeted physical examination will be performed at Day 1, Day 85, and Day 169 as well as the ET visit where applicable.

For the remaining visits, a symptom-driven physical examination will be performed as per the investigators' discretion. Physical examination including details of clinically significant findings, will be listed.

6.7. Columbia-Suicide Severity Rating Scale

The C-SSRS is a questionnaire that prospectively assesses suicidal ideation and suicidal behavior (Posner 2011). The C-SSRS, which uses a semi-structured interview to probe subject responses, is administered by an individual who has received training and certification in its administration. During the Screening Visit (Day -56), the "baseline" version of the C-SSRS is administered. This

version assesses suicidal ideation and suicidal behavior during the subject's lifetime. At all subsequent visits, the "since last visit" version is administered.

Counts and percentage of subjects who experience suicidal ideation, suicidal behavior or neither (no ideation or behavior) will be summarized by treatment group at baseline and double-blind treatment period.

The C-SSR instrument consists of 11 Yes/No questions as shown in the table below and is grouped by type question, i.e., suicidal ideation questions and suicidal behavior questions. Within each type/group the questions are re-ordered by increasing severity. In addition, subjects are assessed for any self-injurious behavior without suicidal intent.

C-SSRS Item	Derivation
Suicidal ideation	A "yes" answer to any one of the following five questions. 1. Wish to be dead. 2. Non-specific active suicidal thoughts. 3. Active suicidal ideation with any methods (not plan) without intent to act. 4. Active suicidal ideation with some intent to act, without specific plan. 5. Active suicidal ideation with specific plan and intent.
Suicidal behavior	A "yes" answer to any one of the following five questions. • Preparatory acts or behavior • Aborted attempt • Interrupted attempt • Actual attempt • Suicidal behavior • Completed suicide
Self-injurious behavior without suicidal intent	A "yes" answer to the following question from suicidal behavior section on the C-SSRS: Has subject engaged in Non-Suicidal Self-Injurious behavior?
Suicidal Ideation Score	Is defined as the maximum suicidal ideation category (1-5 on the C-SSRS) present at the assessment. A score of 0 is assigned if no ideation is present.

For the overall assessment of suicidal ideation and behavior during the double-blind treatment period a subject's worst finding during that period will be used.

The following tables will be generated at the end of the Double-Blind Treatment Period:

- Counts and Percentage of Subjects with Suicidal Ideation, Suicidal Behavior, and Self-Injurious Behavior without Suicidal Intent Based on the C-SSRS by Treatment group at baseline.
- Counts and Percentage of Subjects with Suicidal Ideation, Suicidal Behavior, and Self-Injurious Behavior without Suicidal Intent Based on the C-SSRS by Treatment group during the double-blind treatment period.

- Counts and Percentage of Subjects with Suicide-Related Treatment Emergent Events Based on C-SSRS by Treatment group during the Double-Blind Treatment Period.
- Shift table of changes in C-SSRS categories (ideation, behavior, or neither) from baseline to through Week 24.
- Shift table in Changes in C-SSRS Suicidal Ideation scores from baseline to through Week 24 by Treatment.

All C-SSRS assessments will be listed.

6.8. Other Safety: Body Weight, BMI, Breast Imaging and Pregnancies

Bodyweight
Bodyweight is assessed at Screening, Day 1, Day 85, and Day 169, as well as the ET visit where applicable. Height is assessed once at the screening visit.

The observed bodyweight and BMI values at each visit, along with the change from baseline to each post-baseline visit will be summarized descriptively as continuous variables by treatment group and overall. The following categorizations will additionally be summarized for body weight at each visit:

Category	Change from Baseline
Category 1	≥ 10% loss
Category 2	≥ 5 - 10% loss
Category 3	-5% < Weight change <+5%
Category 4	≥ 5 - 10% increase
Category 5	≥ 10% increase

Breast Imaging and Pregnancies
Breast imaging for female subjects is conducted at the screening visit and at the day 169 visit. Imaging is not required during the screening period if the subject has had a negative breast cancer imaging status (i.e., not considered clinically abnormal and/or requiring further evaluation/treatment) within 6 months prior to the screening visit. If clinically indicated, an ultrasound or MRI may also be conducted for breast cancer imaging, per the investigator's discretion. Summary statistics will be used to describe the breast imaging results.

6.9. Subgroup Analyses for Safety

The count and percentage of subjects who report at least one TEAE will be summarized by treatment group and PT for the subgroups outlined below.

- Sex (male, female)
- Race (White, Non-white)
- Age at baseline (<65, ≥ 65 years)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino)
- Geographic region (North America, Europe, South America, Asia)

- Slow vital capacity (80%, ≥ 80%)
- Use of approved background therapy for ALS (Y/N)

7. PHARMACOKINETIC OUTCOMES

Approximately 36 subjects from selected sites will be enrolled in the PK sub-study.

- Blood samples for riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol PK will be collected at Day -28 predose (prior to the first dose of riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol on Day -28) and 1 hour (±15 minutes) post-dose.
- Blood samples for PTC857, riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol PK will be collected from those subjects taking part in the PK Sub-Study at predose (prior to the first dose of the day) and at 1, 2, 4, 5, 6, 8, and 12 hours (±15 minutes) (prior to the second dose) post-dose on Day 1 and Day 29.
- Blood samples for PTC857 PK only will be collected predose (prior to the first dose of the day) and at 1 hour (±15 minutes) and 4 hours (±1 hour) post-dose on Day 85, and predose only at all subsequent visits with blood PK sampling.
- CSF samples will be collected predose (first dose of the day) on the Baseline Visit, Day 29 Visit, and the End of Treatment Visit.

Summary statistics for plasma concentrations vs. time will be provided, in addition to by-subject listings.

PK data collected from this study will be included in non-compartmental analysis and Population PK analysis. Separate PK analysis plan(s) and report(s) will be provided.

8. REFERENCES

Group, WGEM-AS. Safety and efficacy of edaravone in well-defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2017; 16(7): 505-512.

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 Apr;14(3):162-8. doi: 10.3109/21678421.2012.762930. Epub 2013 Jan 17. PMID: 23323713.

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, Nakanishi A. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999 Oct 31;169(1-2):13-21. doi: 10.1016/s0022-510x(99)00210-5. PMID: 10540002.

Norris FH, Calanchini PR, Fallat RJ, Panchari S, and Jewett B. The administration of guanidine in amyotrophic lateral sclerosis. Neurology, Vol 24 | Number 8, August 01, 1974.

Jenkinson C, Fitzpatrick R, Brennan C, Bromberg M, Swash M. Development and validation of a short measure of health status for individuals with amyotrophic lateral sclerosis/motor neurone disease: the ALSAQ-40. J Neurol. 1999 Nov;246 Suppl 3:III16-21. doi: 10.1007/BF03161085. PMID: 10631656.

Garcia-Hernandex, A and Rizopoulos, D. %JM: A SAS Macro to Fit Jointly Generalized Mixed Models for Longitudinal Data and Time-to-Event Responses. Journal of Statistical Software. 2018 Apr; 84(12), doi:10.18637/jss.v084.i12.

Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17:423-433

van Eijk RPA, Westeneng HJ, Nikolakopoulos S, et al. Refining eligibility criteria for amyotrophic lateral sclerosis clinical trials. Neurology 2019;92:451-460

van Eijk RPA, Roes KCB, van den Berg LH, Lu Y. Joint modeling of endpoints can be used to answer various research questions in randomized clinical trials. J Clin Epidemiol. 2022 Jul;147:32-39. doi: 10.1016/j.jclinepi.2022.03.009. Epub 2022 Mar 26. PMID: 35346783.

TRICALS Eligibility, Standard Operating Procedure, version 1.0, 2020, https://tricals.shinyapps.io/risk-profile/

van Eijk RPA, et al. Innovating Clinical Trials for Amyotrophic Lateral Sclerosis. Challenging the Established Order, Neurology Sep 2021, 97 (11) 528-536; DOI: 10.1212/WNL.0000000000012545

Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17:423-433

Rubika Balendra, Ashley Jones, Naheed Jivraj, Catherine Knights, Catherine M. Ellis, Rachel Burman, Martin R. Turner, P. Nigel Leigh, Christopher E. Shaw & Ammar Al-

Chalabi (2014) Estimating clinical stage of amyotrophic lateral sclerosis from the ALS Functional Rating Scale, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 15:3-4, 279-284, DOI: 10.3109/21678421.2014.897357

Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, Currier GW, Melvin GA, Greenhill L, Shen S, Mann JJ. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry. 2011 Dec;168(12):1266-77. doi: 10.1176/appi.ajp.2011.10111704. PMID: 22193671; PMCID: PMC3893686.

Paganoni S., et al.; N Engl J Med. 2020 Sep 3;383(10):919-930. doi: 10.1056/NEJMoa1916945.; Trial of Sodium Phenylbutyrate-Taurursodiol for Amyotrophic Lateral Sclerosis.

Writing Group; Edaravone (MCI-186) ALS 19 Study Group; Lancet Neurol.,2017 Jul;16(7):505-512. doi: 10.1016/S1474-4422(17)30115-1.; Safety and efficacy of edaravone in well-defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial.

9. PROGRAMMING CONSIDERATIONS

All tables, figures, listings (TFLs), and statistical analyses will be generated using SAS for Windows, Release 9.4 or higher (SAS Institute Inc., Cary, NC, USA). Computer-generated table, listing and figure output will adhere to the following specifications.

1. One SAS program can create several outputs.
2. Each output will be stored in a separate file.
3. Output files will be delivered in Word format.
4. The numbering of TFLs will follow ICH E3 guidance.
5. All tables and listings will be produced in landscape orientation. Figures use the orientation that best facilitates interpretation of the data.
6. All TFLs will be produced using the Courier New font, size 10. The smallest acceptable point size for the Regulatory Authorities is 8.
7. Headers and footers for figures will be in Courier New font, size 8, which is the smallest acceptable point size for the Regulatory Authorities.
8. TFLs will be in black and white (no color), unless otherwise specified.
9. Specialized text styles, such as bolding, italics, borders, shading, and color are not used in the tables and listings, unless otherwise specified. Color may be used in figures containing multiple plotted lines as long as information is not lost when the figure is reproduced in monotone.
10. A mixed case will be used for titles, footnotes, column headers, and programmer-supplied formats, as appropriate.
11. For categorical parameters, all categories will be presented in the table, even if n=0 for all treatment groups in each category. For example, the frequency distribution for symptom severity would appear as:

Severity Rating	n
Mild	2
Moderate	5
Severe	0

- An Unknown or Missing category will be added to each parameter for which information is not available for 1 or more subjects.
- Unless otherwise specified, the estimated mean, median, Q1 and Q3 for a set of values will be printed out to 1 more significant digit than the original values, and standard deviations and standard errors will be printed out to 2 more significant digits than the original values. The minimum and maximum will report the same significant digits as the original values.
- Confidence intervals are displayed within round brackets and separated by a comma. The lower limit is presented first, and the upper limit is presented second. The confidence interval is placed either beside or below the corresponding estimate. The estimate and the confidence limits are presented using the same degree of precision.

- P-values will be output in the format: '0.xxx', where xxx is the value rounded to 3 decimal places. Every p-value less than 0.001 will be presented as <0.001. If the p-value is returned as >0.999, then present as >0.999.
- Percentage values will be printed to one decimal place. Unless otherwise noted, for all percentages, the number of subjects in the analysis population for the treatment group who have an observation will be the denominator. Percentages after zero counts will not be displayed and percentages equating to 100% will be presented as 100%, without decimal places.
- Tabular display of data for medical history, prior/concomitant medications, and all tabular displays of adverse event data will be presented by the body system, treatment class, or SOC with the highest occurrence in the active treatment group in decreasing order, assuming all terms are coded. Within the body system, drug class and SOC, medical history (by preferred term), drugs (Medication class), and adverse events (by preferred term) will be displayed in decreasing order. If incidence for more than 1 term is identical, they will then be sorted alphabetically.
- Missing descriptive statistics or p-values which cannot be estimated will be reported as '-' Missing values for numeric and character variables are displayed as blanks in data listings.
- The percentage of subjects will normally be calculated as a proportion of the number of subjects assessed in the relevant treatment group (or overall) for the analysis population presented. However, careful consideration is required in many instances due to the complicated nature of selecting the denominator, usually the appropriate number of subjects exposed. Details will be described in footnotes or programming notes, as necessary.
- If no relevant data are available at all for a display, then present "No data to Report".

10. MOCK TABLES, LISTINGS, AND GRAPHS

The study tables, listings and graphs shells will be provided in a separate document.

11. APPENDIX 1: SCHEDULE OF ASSESSMENTS

Schedule of Assessments for Screening and Double-Blind Treatment Period (Part A)

			Treatment Period (24 W)								EOS/ FU	Early Termination
Study Day	**Screening (-56 Days) (±5)**	**Day -28 (±3)**	**Day 1 (-3)[a]**	**Day 14 (±3)**	**Day 29 (±3)**	**Day 57 (±3)**	**Day 85 (±3)**	**Day 113 (±3)**	**Day 141 (±3)**	**Day 169 (±3)**	**Day 197 (±3)**	
			Baseline[a]							**End of Treatment[b]**	**Follow-Up[c]**	**ET[d]**
Telephone call/in-home or in-person visit[f]	IP	PC	IP	PC/IH	PC/IH[e,f]	PC/IH[f]	IP	PC/IH[f]	PC/IH[f]	IP	PC	IP
COVID-19 PCR	X											
Informed consent	X						X[g]			X[h]		
Inclusion/exclusion criteria	X											
Re-evaluation eligibility			X									
Randomization			X									
Height	X											
Weight and BMI	X		X				X			X		X
Demographics	X											
Medical history	X											
Serum/urine pregnancy test (females only)[i]	X		X		X	X	X	X	X	X	X	X
FSH measurement[j]	X											
12-Lead ECG[k]	X		X				X			X		X
Vital signs[l]	X		X		X	X	X	X	X	X		X
Physical exam[m]	X		X				X			X		X
Sparse PK blood sampling[n]			X				X			X		X
Clinical laboratory tests[o]	X		X	X[p]	X	X	X			X		X
CSF for biomarkers[q,r]			X							X		X
Laboratory biomarkers (blood and urine)[s]			X				X			X		X
ALSFRS-R[t]	X	X	X		X	X	X	X	X	X	X	X
PFTs[u]	X		X				X			X		X
ALS CBS	X		X				X			X		X
Modified Norris Scale			X				X			X		X
ALSAQ-40			X				X			X		X
EQ-5D-5L			X				X			X		X
C-SSRS[v]	X	X	X		X	X	X	X	X	X	X	X
Study drug dispensation			X				X					
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Prior/concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
Mammogram[w]	X									X		X[x]

			Treatment Period (24 W)								EOS/ FU	Early Termination
Study Day	**Screening (-56 Days) (±5)**	**Day -28 (±3)**	**Day 1 (-3)[a]**	**Day 14 (±3)**	**Day 29 (±3)**	**Day 57 (±3)**	**Day 85 (±3)**	**Day 113 (±3)**	**Day 141 (±3)**	**Day 169 (±3)**	**Day 197 (±3)**	
			Baseline[a]							**End of Treatment[b]**	**Follow-Up[c]**	**ET[d]**
PK Sub-Study Assessments[y]												
Telephone call or in-person visit	IP	IP	IP	PC/IH	IP	PC/IH	IP	PC	PC	IP	PC	IP
Serial PTC857 PK blood sampling			X		X		X			X		X
Riluzole PK blood sampling		X	X		X							
Edaravone PK blood sampling		X	X		X							
Sodium phenylbutyrate/taurursodiol PK sampling		X	X		X							
CSF for PK[z]			X		X					X		X
Additional ECGs			X		X							

Abbreviations: ALS, amyotrophic lateral sclerosis; ALS CBS, ALS Cognitive Behavioral Screen; ALSAQ-40, ALS Assessment Questionnaire; ALSFRS-R, ALS Functional Rating Scale-Revised; BMI, body mass index; COVID-19, coronavirus disease 2019; CSF, cerebrospinal fluid; C-SSRS, Columbia-Suicide Severity Rating Scale; ECG, electrocardiogram; EOS, end of study; EQ-5D-5L, five-level European quality of life five-dimensional questionnaire; ET, early termination; FSH, follicle-stimulating hormone; FU, follow-up; ICF, Informed Consent Form; IH, in-home; IP, in-person; LTE, Long-Term Extension; p75NTR, p75 neurotrophin receptor; PC, telephone call; PCR, polymerase chain reaction; PFT, pulmonary function test; PI, Principal Investigator; PK, pharmacokinetic; SAE, serious adverse event

[a] The Baseline Visit will be defined as Day 1 of the study. Baseline Visit procedures may be performed within the 3 days before the day that study drug is first administered. The day in which the first dose of study drug is administered will be Day 1. For subjects taking part in the PK Sub-Study, the PK laboratory tests must be completed on the same day as the first dose of study drug.
[b] Day 169 will be considered the End of Treatment Visit only for those who choose not to enter the LTE Period (Part B).
[c] Follow-up Visit on Day 197 only for subjects who choose not to enter the LTE Period (Part B).
[d] For subjects who terminate early from the study and consent to continued follow-up, the investigator should attempt to gain vital status on the participant and conduct the ALSFRS-R assessment approximately every 28 days for the remaining duration of the study, in accordance with local requirements/regulations.
[e] The Day 29 Visit will be conducted in-person for subjects participating in the PK Sub-Study only.
[f] For the telephone call/in-home visits, where applicable, the ALSFRS-R, C-SSRS, adverse events, and prior/concomitant medications assessments will be conducted via a telephone call. Vital signs assessments and samples for clinical laboratory tests will be collected via a home health nurse during applicable in-home visits at the subject's home during these visits. Subjects may go to the site for an in-person visit at the discretion of the PI. Visits indicated as in-person only may be conducted remotely (ie, in-home) following discussion between the site and the medical monitor. In-person visits that are conducted remotely must include the following assessments, at minimum (if such assessments are typically included during the visit): serum/urine pregnancy test (females only), vital signs, clinical laboratory tests, ALSFRS-R, C-SSRS, adverse events, prior/concomitant medications, sparse PK blood sampling, laboratory biomarkers, and ALSAQ-40.
[g] The ICF for Part B should be provided to the subject at the Day 85 visit to allow the subject adequate time to consider transitioning into Part B.
[h] The ICF for Part B should be signed by the subject at the Day 169 visit if they choose to enroll into Part B.
[i] Women of childbearing potential will have a serum pregnancy test at the initial Screening Visit and then undergo monthly urine pregnancy testing starting from Day 1 prior to randomization through the Follow-up Visit. If a urine pregnancy test is positive, a serum pregnancy test will be conducted for confirmation and dosing should be immediately suspended and evaluated for continued study participation based upon the result of the serum pregnancy test and in discussion with the medical monitor. For months where an on-site visit is not scheduled, subjects will complete a urine pregnancy test at home, if local requirements allow, to be reported to the investigator and documented in the eCRF.
[j] Postmenopausal female subjects only.
[k] Single 12-lead ECG measurements will always be performed prior to the dose of medication administered at the clinic. Subjects should be resting for at least 5 minutes in supine position prior to 12-lead ECG procedures.
[l] Vital signs will consist of measurements of supine blood pressure, heart rate, body temperature, and respiratory rate. Vital sign measurements will be collected after the subject has been resting for 5 minutes. Blood pressure and heart rate measurements will be performed with subjects in a supine position, except when they semi reclined because of study procedures and/or AEs (eg, nausea, dizziness) or if deemed necessary by the PI or designee.
[m] A full physical examination will be completed during Screening Period. A targeted physical examination, including neurological examination, will be completed at all other timepoints.
[n] Blood samples for PK evaluation will be collected from all subjects not participating in the PK Sub-Study at predose (prior to the first dose of the day) and at 1 hour (±15 minutes) and 4 hours (±1 hour) post-dose on Day 1 and Day 85. The first 36 subjects that enroll in the study will have a 6-hour (±1 hour) post-dose blood sampling on Day 1 and Day 85; this 6-hour post-dose blood sampling must be at least 2 hours following the 4-hour post-dose blood sampling. Subjects enrolled after the first 36 subjects may optionally have the 6-hour (±1 hour) post-dose blood sampling on Day 1 and Day 85, if feasible. Time of last dose of study drug prior to the study visit should be recorded. On all other visits with blood samples for PTC857 PK, blood samples will only be collected predose. If any SAEs are observed, ad hoc PK sample(s) may be collected, if possible, to measure the levels of PTC857, riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol to determine the causality.
[o] Clinical laboratory tests will consist of fasting lipid profile, hematology, coagulation, blood chemistry, and urinalysis. Urinalysis will only be performed at in-person clinic visits (ie, Screening, Day 1, Day 85, and Day 169).
[p] Clinical laboratory tests on Day 14 will consist only of a fasting lipid profile. Hematology and blood chemistry will not be evaluated during this visit.
[q] It is the intention of the protocol that all subjects will consent to undergo lumbar punctures. Cerebrospinal fluid samples for biomarkers and PK will be collected at predose (first dose of the day) on both the Baseline Visit and the Day 169 Visit; lumbar punctures on these days may be conducted up to 5 days prior to the Baseline Visit and the Day 169 Visit. CSF biomarkers will be assessed as listed in Section 7.1.7.
[r] The lumbar puncture that is required may be skipped for an individual subject if the PI deems it appropriate, and after discussion with the medical monitor.
[s] Laboratory (blood and urine) biomarkers (aligned with the timing for the clinical laboratory tests) as per Section 7.1.7. Urine creatinine should be tested when urine p75NTR is sampled.
[t] The ALSFRS-R assessment must be performed by a certified rater.
[u] Pulmonary function tests will assess slow vital capacity and Sniff Nasal Inspiratory Pressure. Forced vital capacity, vital capacity, forced expiratory volume, and maximum inspiratory pressure will not be measured. Refer to the laboratory manual for specific requirements at Screening. Oxygen saturation, respiratory rate, and heart rate will be assessed in both upright and supine positions. The Sniff Nasal Inspiratory Pressure test will assess nasal pressure in each unilaterally occluded nostril during a maximal sniff to assess inspiratory muscle strength.
[v] During the Screening Visit (Day -56), the "baseline" version of the C-SSRS will be administered. At all other visits, the "since last visit" version will be administered.
[w] Mammograms to be conducted for female subjects only. A mammogram is not required during the Screening Period if the subject has had a negative breast cancer imaging status (ie, not considered clinically abnormal and/or requiring further evaluation/treatment) within 6 months prior to the Screening Visit. If clinically indicated, an ultrasound or MRI may also be conducted for breast cancer imaging, per the investigator's discretion. If a subject cannot complete the Screening mammogram or any necessary follow-up procedures during the Screening Period, the Screening Period may be extended after discussion and agreement with the medical monitor.
[x] Women who have been exposed to study drug for at least 3 months and discontinue treatment before reaching the Day 169 Visit will have breast cancer surveillance imaging performed at the Early Termination Visit. Women who have been exposed to study drug for <3 months and discontinue treatment before reaching the Day 169 Visit may have breast cancer surveillance imaging performed at the investigator's discretion.
[y] The following additional procedures will be conducted only in subjects taking part in the optional PK Sub-Study:

- Blood samples for riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol PK will be collected at Day -28 predose (prior to the first dose of riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol on Day -28) and 1 hour (±15 minutes) post-dose. Subjects should be instructed not to take riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol on Day -28 prior to the study visit.

- Blood samples for PTC857, riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol PK will be collected from those subjects taking part in the PK Sub-Study at predose (prior to the first dose of the day) and at 1, 2, 4, 5, 6, 8, and 12 hours (±15 minutes) (prior to the second dose) post-dose on Day 1 and Day 29. Blood samples for PTC857 PK only will be collected predose (prior to the first dose of the day) and at 1 hour (±15 minutes) and 4 hours (±1 hour) post-dose on Day 85, and predose only at all subsequent visits with blood PK sampling. Subjects should be instructed not to take riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol on Day 1 or Day 29 prior to the study visit. Time of last dose of study drug and riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol prior to the study visit should be recorded.
- Additional samples of predose (trough) CSF will be collected for PK evaluation at all lumbar puncture timepoints.
- Additional ECG measurements will be performed at the time of the 4-hour and 8-hour post-dose PK samples (±15 minutes from PK sample blood draw).

[z] The lumbar punctures for CSF collection in the PK Sub-Study may occur within 5 days prior to or after the scheduled visit day.

Schedule of Assessments for Long-Term Extension Period (Part B)

Schedule of Assessments for Continued Long-Term Extension Period (Part C)

12. APPENDIX 2: MISSING DATE AND MISSING ALSFRS-R PROCEDURE

Prior/Concomitant Medications

For the purpose of assessing whether a medication is prior or concomitant, if a medication has a completely missing start date it will be considered a prior medication, and if a medication has a completely missing stop date it will be considered a concomitant medication. If a partial start or stop date occurs, the following imputation process will be implemented:

Partial Missing Start or Stop Date	Imputation for Start Date	Imputation for Stop Date
Day missing, month and year present	• Month and/or year different to month and year of first study drug dose: Impute day with "01" • Month and/or year same as month and year of first study drug dose: Impute day with same day as first dose of study drug.	Impute day with last day of the month
Day and month missing, year present	• Year different to year of first study drug dose: Impute day and month with "01JAN" • Year same as year of first study drug dose: Impute month and day with same month and day as first dose of study drug.	Impute day and month with "31DEC"
Month missing, day and year present	• Year different to year of first study drug dose: Impute month with "JAN" • Year same as year of first study drug dose: Impute month with same month as first dose of study drug.	Impute with "DEC"
Caveats	• If any imputed start date leads to a start date that is after the stop date, then the start date will be imputed with the date of the stop of medication. • No stop date will be imputed if the treatment is ongoing.	

Adverse Events

For the purpose of assessing whether an AE is treatment emergent, if an AE has a completely missing start and stop date, it will be considered treatment emergent; if the stop date is not missing, but the start date is completely missing, it will be considered treatment emergent unless the stop date occurs prior to the first dose of study drug.

For assessing treatment emergence or for calculation of duration of AE, if a partial start or stop date occurs, the following imputation process will be implemented:

Partial Missing Start or Stop Date	Imputation for Start Date	Imputation for Stop Date
Day missing, month and year present	• Month and/or year different to month and year of first study drug dose: Impute day with "01". • Month and/or year same as month and year of first study drug dose: Impute day with same day as first dose of study drug.	Impute day with last day of the month
Day and month missing, year present	• Year different to year of first study drug dose: Impute day and month with "01JAN". • Year same as year of first study drug dose: Impute month and day with same month and day as first dose of study drug.	Impute day and month with "31DEC"

Partial Missing Start or Stop Date	Imputation for Start Date	Imputation for Stop Date
Month missing, day and year present	• Year different to year of first study drug dose: Impute month with "JAN". • Year same as year of first study drug dose: Impute month with same month as first dose of study drug.	Impute with "DEC"
Caveats	• If any imputed start date leads to a start date that is after the stop date, then the start date will be imputed with the date of the stop of AE.	

ALSFRS-R incomplete / missing data

This section describes visit level imputations performed prior to the multiple imputation procedure.

If 1 item is missing within a domain (e.g. Bulbar), then the domain item will be inputted using the following formula:

Imputed domain score = mean of non-missing items multiplied by 3.

If item 12 on the ALSFRS score is missing, and the patient is not reported as having received non-invasive ventilation at the time of the visit (concomitant procedures CRFs) then item 12 will be imputed as a 4.

Total Score:

If a whole domain is missing or two items are missing, then the total ALSFRS-R total score will be imputed from the remaining 3 domains as the mean of all answered item scores in the 3 domains x 12. Total score will not be imputed if more than 1 domain has 2 or more missing items.

13. APPENDIX 3: TRICALS RISK PROFILE CALCULATOR

TRICALS Risk Profile Calculator, v2.01 (2022-02-20), Source: https://tricals.shinyapps.io/risk-profile/

The TRICALS Risk Profile is a prognostic summary value of seven patient characteristics and can be used for patient selection in clinical trials. The Risk Profile Calculator estimates the TRICALS Risk Profile.

References

1. Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17:423-433
2. van Eijk RPA, Westeneng HJ, Nikolakopoulos S, et al. Refining eligibility criteria for amyotrophic lateral sclerosis clinical trials. Neurology 2019;92:451-460
3. van Eijk RPA, Nikolakopoulos S, Roes KCB et al. Innovating clinical trials for amyotrophic lateral sclerosis: challenging the established order. Neurology 2021

Estimation of the TRICALS Risk Profile

The TRICALS Risk Profile is based the ENCALS (European Network to Cure ALS) survival prediction model, a cross-validated model to predict the composite endpoint death or respiratory insufficiency based on data of 11,475 patients with ALS. Patient selection is based on the linear predictor of the model and not on the absolute risk predictions as (1) the baseline hazard significantly varies across countries, and (2) the ENCALS model estimates the absolute survival probability from disease onset rather than trial enrolment. The linear predictor of the model, hereafter stated as TRICALS Risk Profile, can be conceptualized as a relative summary of prognostic information. The risk profile indicates how patients compare to each other (i.e. who is faster or slower progressing than average) without estimating the absolute survival time or probability.

The TRICALS Risk Profile is calculated as:

$$\boldsymbol{Risk\ profile} = (0.474 \times VC_t) - (2.376 \times DxDelay_t) - (1.839 \times \Delta FRS_t) - (0.264 \times Age_t) + (0.271 \times Bulbar) + (0.238 \times Definite) + (0.415 \times FTD)$$

Where *t* represents the non-linear transformations for continuous variables, VC the vital capacity (in %predicted), Age is age at onset in years, diagnostic delay (DxDelay) in months, FTD the presence of frontotemporal dementia and ΔFRS (points per month) is the progression rate of the ALS functional rating scale (ALSFRS-R).

$$Age_{transf.} = \left(\frac{Age\ at\ onset}{100}\right)^{-2} \qquad DxDelay_{transf.} = \left(\frac{DxDelay}{10}\right)^{-\frac{1}{2}} + \ln\left(\frac{DxDelay}{10}\right)$$

$$\Delta FRS_{transf.} = (\Delta FRS + 0.1)^{-\frac{1}{2}} \qquad VC_{transf.} = \left(\frac{VC}{100}\right)^{-1} + \left(\frac{VC}{100}\right)^{-\frac{1}{2}}$$

$$\Delta FRS_{transf} = \left(48 - FRS_{screening}\right) \div DxDelay$$

14. APPENDIX 4: SAS CODE

Mixed Model Repeated Measure

The following is example SAS syntax for producing least squares estimates, for the MMRM analysis:

```
proc mixed data=ALSFRS_LONG method=reml;
   class ID TRTRND(ref="0") STADCARE(ref="3") CHANGE(ref="<1")
TIME(ref="baseline");
   model DELFRS = TIME VALUEBL CHANGE STADCARE TIME*TRTRND TIME*VALUEBL TRP
   TIME*TRP / ddfm=kr solution;
   repeated TIME / type=UN subject=id;
   lsmeans trtrnd*TIME /cl pdiff=control('0' '5')cl;
   run;
```

where `id` is the subject identifier, `trtrnd` is the randomized treatment arm, `stadcare` is a factor variable for the SoC stratification group at baseline, `change` is the amount of change in the subject's ALSFRS-R score during the Screening Period, `time` is the categorical variable for study visit, `Valuebl` is the baseline ALSFRS-R score, `TRP` represents the TRICALS risk profile at baseline, and the response variable `DELFRS` is the change from baseline in ALSFRS-R score.:

ANCOVA with Multiple Imputation

SAS code for the ALSFRS-R endpoint at 24 weeks:

```
proc mi data = ALSFRS_DATA out = ALSFRS_DATA_OUT
   seed = 20221219 nimpute = 100 noprint;
   class trtrnd stadcare change sex;
   var trtrnd Valuebl change stadcare sex age trp
   ALSFRS_R_4 ALSFRS_R_8 ALSFRS_R_12 ALSFRS_R_16 ALSFRS_R_20 ALSFRS_R_24;
   fcs reg(ALSFRS_R_4 ALSFRS_R_8 ALSFRS_R_12 ALSFRS_R_16 ALSFRS_R_20
   ALSFRS_R_24);
   run;
```

The next set of code performs the analyses for the ALSFRS-R 24-week endpoint:

```
proc reg data=ALSFRS_DATA_OUT  utset=outreg covout noprint;
   model ALSFRS_24 = trtrnd Valuebl change stadcare TRP;
   by _Imputation_;
   run;
```

This step will combine the results:

```
proc mianalyze data=outreg;
   modeleffects Intercept trtrnd Valuebl change stadcare TRP;
   run;
```

Pattern Mixture Model

The strategy implemented to carry out the control-based PMM assumes that all other variables, except ALSFRS-R, with missing data are first imputed using the FCS approached outlined in the previous sensitivity analysis, with 100 imputation datasets. These data are stored in DATAIN_IMP0, which contains a variable _Imputation_ to distinguish between multiple copies of the original input data. In the next step missing ALSFRS-R at the first time point will be imputed (the first time-point that has some missing data). To use the control-based imputation

method, the starting dataset will be separated into two separate datasets: DATAIN_IMP1, containing all Placebo subjects and those subjects from the PTC875 arm that have values at time-point 1 missing; and DATAIN_REST1, containing the remainder of the subjects from the PTC875 arm who have ALSFRS-R scores available at this time point.

Create a variable VISIT1 where it is equal to 1 if ALSFRS-R is available at this visit and 0 if it is missing.

```
Data DATAIN_IMP1 DATAIN_REST1;
  set DATAIN_IMP0;
  if trtrnd = 1 and VISIT1 = 1 then output DATAIN_REST1;
  else output DATAIN_IMP1;
  run;
```

Next, use PROC MI to impute missing data at the first timepoint with missing data, based on the model estimated exclusively from control subjects with non-missing values at time-point 1. The following code assumes that Week 4 contains the first missing data from the ALSFRS-R outcome dataset.

```
Proc mi data= DATAIN_IMP1 out=DATAIN_REG_IMP1 nimpute=1 seed=1001;
      class trtrnd stadcare change sex;
      by _Imputation_;
      var trtrnd Valuebl change stadcare sex age trp ALSFRS_R_4;
      monotone reg(ALSFRS_R_4);
      run;
```

Next, combine all the data into one dataset.

```
Data DATAIN_IMP1_COMB;
  set DATAIN _REST1 DATAIN_REG_IMP1;
  run;
```

Repeat the above steps for the second timepoint with missing data for ALSFRS-R. Dataset DATAIN_IMP1_COMB will be used for input to the next PROC MI call, but first, separate it into two datasets: DATAIN _IMP2, containing all Placebo subjects and those subjects from the experimental arm that have values at second timepoint missing; and DATAIN_ REST2, containing the remainder of the subjects from the PTC875 arm. Create a variable VISIT2 where it is equal to 1 if ALSFRS-R is available at the second timepoint with missing data and 0 if it is missing. The below code assumes that this will be at Week 8.

```
Data DATAIN_IMP2 DATAIN_REST2; set DATAIN_IMP1_COMB;
      if trtrnd = 1 and VISIT2 = 1 then output DATAIN_REST2;
      else output DATAIN_IMP2;
      run;
```

Next use PROC MI to impute missing data at time-point 2 based on the model estimated exclusively from Placebo subjects with non-missing data at time-point 2.

```
Proc sort data= DATAIN_IMP2; by _Imputation_; run;
proc mi data=DATAIN_IMP2 out=DATAIN_REG_IMP2 nimpute=1 seed=1002;
      by _Imputation_;
      var trtrnd Valuebl change stadcare sex age trp ALSFRS_R_4 ALSFRS_R_8;
      monotone reg(ALSFRS_R_8);
      run;
```

Re-assemble the data back into a single dataset containing all subjects:

```
data DATAIN_IMP2_COMB;
```

```
set DATAIN_REST2 DATAIN_REG_IMP2;
run;
```

Repeat the same procedure until ALSFRS-R is imputed at all timepoints.

The next set of code performs the analyses for the ALSFRS-R 24-week endpoint as for the FCS multiple-imputation approach, assuming that there were six timepoints at which there were missing data in the ALSFRS-R dataset:

```
proc reg data= DATAIN_IMP6_COMB utset=outreg covout noprint;
   model ALSFRS_24 = trtrnd Valuebl change stadcare TRP;
   by _Imputation_;
   run;
```

This step will combine the results:

```
proc mianalyze data=outreg;
   modeleffects Intercept trtrnd Valuebl change stadcare TRP;
   run;
```

Survival Analysis

The following is sample code for producing a Kaplan-Meier survival plot:

```
proc lifetest data=survdata plots=survival(cl cb=hw test atrisk);
    time time*status(0);
    strata trtrnd / test=logrand adjust=sidak;
    run;
```

where `time` is the time of event or censoring, `status` is the variable indicating censored (1) or not censored (0), i.e., death observed, `trtrnd` is the treatment allocation variable. The following sample code runs a Cox regression model:

```
proc phreg data=survdata atrisk plots(cl)=(survival cumhaz);
   class trtrnd stadcare TRP(ref='B');
   STRATA stadcare change;
   model time*status(0)= trtrnd Valuebl / ties=breslow rl;
   hazardratio trp;
   run;
```

where `time` is the time of event or censoring, `status` is the variable indicating censored (1) or not censored (0), i.e., death observed, and `trtrnd` is the treatment allocation variable.

15. APPENDIX 5. MAPPING ALSFRS-R TO KING'S STAGE

The King's clinical staging criteria for ALS (Balendra 2014) is defined as a count of involved central nervous system (CNS) regions (Stage 1, 2 or 3), requirement for gastrostomy or noninvasive ventilation (Stage 4) and death (Stage 5) .

Stage	Definition
1	Functional involvement of one CNS region (symptom onset)
2	Functional involvement of two CNS regions
3	Functional involvement of three CNS regions
4	Need for gastrostomy (4A) or non-invasive ventilation (4B)
5	Death

1. Bulbar region is involved: If the subject dropped any points on any of the three ALSFRS-R questions regarding speech (question 1), salivation (question 2) or swallowing (question 3).
2. Upper limb region is involved: If the subject dropped any points on either of the two ALSFRS-R questions regarding hand function, which are handwriting (question 4) and ability to cut food and handle utensils (question 5A).
3. Lower limb region is involved: If the subject dropped any points on the ALSFRS-R question regarding ability to walk (question 8).
4. Stage 4A attained: If question 5B is answered instead of question 5A, which is only answered by patients without a gastrostomy.
5. Stage 4B reached: If a patient scored 0 points on question 10 or less than 4 points on question 12.

16. REVISION HISTORY

The following list summarizes the major changes incorporated into Version 2.0 of the SAP from Version 1.0.

Section	Description of Change	Brief Rationale
SAP	The version number and date were updated throughout. The intent of the SAP was changed from a description of all the analyses for the whole study (all Parts) to a description of the analyses pertaining to the double-blind treatment period (Part A). A description of all analyses for the long-term extension periods and entire study are now described in a separate SAP Editorial and administrative revisions (e.g., typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity.	Update
1.2	Objectives for the Long-Term Extension periods (Parts B and C) were removed	Analyses of the Long-term extension periods are described in a separate SAP
1.3.2	Added 2 secondary endpoints	Following Protocol amendment 6.0
1.3	Long-Term Extension periods (Parts B and C) endpoints removed	Analyses of the Long-term extension periods are described in a separate SAP
1.5	Description of sample-size updated following change in the primary endpoint as recommended by the FDA.	Update
1.6	Removed extraneous unblinding details that are not necessary in the SAP, as they are described in the protocol	Update
2.4	Criteria for removal of subjects from the ITT population, to form the per-protocol population is added.	Update
2.6	Added more text with examples of major protocol deviations (PD), and definition of PDs	Update
3.2	Treatment compliance section removed, because it's described in Section 4.8	Update
3.3	The whole section has been updated to reflect FDA feedback, to change the primary efficacy endpoint from change from baseline in ALSFRS-R to subject ranks based on the combined assessment of function and survival (CAFS). Text updated to provide more clarity	Update
4.8	Treatment compliance is more clearly defined, and formula added. Extent of exposure is more clearly defined.	Update
4.9	Visit Windows for The Early Termination Visit and Unscheduled Visits were added.	
5	The whole section has been updated to reflect FDA feedback, to change the primary efficacy endpoint from change from baseline in ALSFRS-R to CAFS. Text updated to provide more clarity. T Joint Model (Shared parameter) analysis removed.	Update Joint model requires making unknown assumptions regarding the change in function overtime.
5.6	Additional subgroup analyses of interest added	Update

Section	Description of Change	Brief Rationale
6.2.1	Additional incidence rate analyses specified	Update
6.2.2	Additional summaries to evaluate differences in AEs that are indicative of disease progression added	Update
6.2.3	Additional summaries to evaluate disproportionate rate of disease progression by treatment	Update
6.8	Weight change categories added Breast Imaging and Pregnancy summaries specified	Update
6.9	Subgroup Analyses for Safety specified	Update

ERRATUM

STATISTICAL ANALYSIS PLAN
FOR THE DOUBLE-BLINDED TREATMENT PERIOD (PART A) OF PTC857-CNS-001-ALS

VERSION NUMBER: 2.0, DATED: 25 SEPTEMBER 2024

ISSUE DATE: 29 OCTOBER 2024

The purpose of this erratum is to correct a typographical error in the Statistical Analysis Plan for Part A of Study PTC857-CNS-001-ALS, version 2.0, dated: 25 SEPTEMBER 2024.

The first paragraph of Section 5.1 contains redundant text implying that the TRICALS variable would be incorporated into the model twice (as a covariate and as a main effect), instead of solely intended as a covariate. The following table displays the paragraph with the extraneous text marked in strikethrough font.

The primary efficacy variable, subject ranks based on the combined assessment of function and survival (CAFS) after 24 weeks of treatment will be performed using an analysis of covariance model (ANCOVA) following multiple imputation. The estimand framework described in Section 3.3.1 will be used. The ANCOVA model will contain the following main effects: treatment (PTC857 or placebo), the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2, or 3-4-points total loss), ~~TRICALS risk profile (continuous),~~ use of Approved Background Therapy for ALS (Yes [either riluzole, edaravone, or sodium phenylbutyrate/taurursodiol], No). Baseline ALSFRS-R score (continuous) and The TRICALS risk profile (continuous) will be included as covariates.

STATISTICAL ANALYSIS PLAN

A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Parallel Study to Assess the Efficacy, Safety, Tolerability, PK and Biomarker Effects of PTC857 in Adult Subjects with Amyotrophic Lateral Sclerosis (CARDINALS)

FOR THE

OPEN LABEL LONG-TERM EXTENSION PERIOD (PART B), CONTINUED LONG-TERM EXTENSION PERIOD (PART C) AND ENTIRE STUDY PERIOD (PARTS A, B AND C POOLED) OF

PTC857-CNS-001-ALS

VERSION 1.0

DATE OF PLAN: 25 SEPTEMBER 2024

STUDY DRUG: UTRELOXASTAT

PROTOCOL NUMBER: PTC857-CNS-001-ALS

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PTC Therapeutics STATISTICAL ANALYSIS PLAN Approval Signatures

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List of Abbreviations

Abbreviation	Definition
AE	Adverse Event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale - Revised
ALS CBS	ALS Cognitive Behavioral Screen
ATC	Anatomical Therapeutic Chemical
BDRM	Blinded Data Review Meeting
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
CAFS	Combined Assessment of Function and Survival
CSF	Cerebrospinal Fluid
CSR	Clinical Study Report
C-SSRS	Columbia-Suicide Severity Rating Scale
DB	Double-Blind
eGFR	Estimated Glomular Filtration Rate
ENR	All Enrolled
ICF	Informed Consent
ITT	Intention to Treat
LFT	Liver Function Test
LTE	Long-term extension
MAR	Missing At Random
MedDRA	Medical Dictionary for Regulatory Activities
NfL	Neurofilament light chain
PAV	Permanent Assisted Ventilation
PFT	Pulmonary Function Test
PK	Pharmacokinetics
PMM	Pattern-Mixture Model
PP	Per Protocol
PT	Preferred Term
SAP	Statistical Analysis Plan
SD	Standard Deviation
SNIP	Sniff Nasal Inspiratory Pressure
SoC	Standard of Care
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
TRICALS	TRICALS is the largest European research initiative to find a cure for ALS
ULN	Upper Limit if Normal
WHO DD	World Health Organization Drug Dictionary

1. INTRODUCTION AND OVERVIEW

The purpose of this statistical analysis plan (SAP) is to provide a description of the statistical methods and procedures to be implemented for data analysis of the open-label long-term extension periods (Parts B and C) and the entire study period (where applicable), for PTC857-CNS-001-ALS, a Phase 2 randomized trial.

This document is based on the final protocol amendment version 6.0, dated 14 JUNE 2024 and has been developed, reviewed and approved prior to the database lock. Any changes from the planned analysis as described in the protocol for PTC857-CNS-001-ALS and its amendments (as applicable) are detailed here, and any differences described here supersede the analysis presented in the protocol. Any additional analyses conducted to supplement the planned analyses and any deviations from the planned analyses described in this SAP will be documented in the Clinical Study Report (CSR).

1.1. Study Design

Brief description of the Study Design

This is a Phase 2, randomized, double-blind, placebo-controlled, parallel-group study to assess the effects of PTC857 (also known as utreloxastat) in adult male and female subjects diagnosed with amyotrophic lateral sclerosis (ALS). Eligible male and female subjects aged between 18 and 80 years will be enrolled in this study. The study consists of 5 periods: Screening, Treatment (Part A), LTE (Part B), Continued LTE (Part C), and Follow Up. During the open-label extension periods (Parts B and C), subjects remain blinded to the original treatment allocation in Part A.

Approximately 340 subjects who meet the inclusion and exclusion criteria will be randomized in a 2:1 ratio to receive PTC857 (250 mg BID) or matching placebo using a central randomization process. The randomization will be stratified for the 2 stratification factors:

1. Amount of change in ALSFRS-R score during the 8-week Screening Period by points total loss:
    a. <1,
    b. 1-2,
    c. 3-4,
    d. >4 points total loss
2. Use of edaravone, sodium phenylbutyrate/taurursodiol, or neither for the treatment of ALS at Screening as standard-of-care therapy:
    a. Edaravone
    b. Sodium phenylbutyrate/taurursodiol
    c. Neither edaravone nor sodium phenylbutyrate/taurursodiol

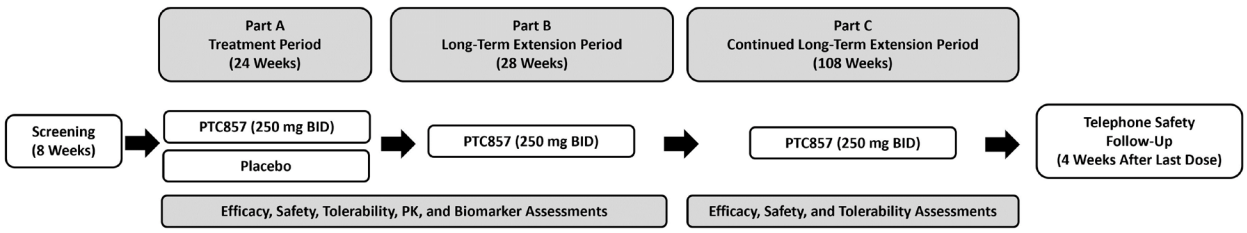
Before protocol amendment version 4.0, dated 31 OCTOBER 2022, the second stratification factor above (treatment for ALS at baseline) was based on use of riluzole alone (i.e., the following two levels: on riluzole therapy for ALS and not on riluzole therapy). Protocol amendment 4.0 opened the study to allow for randomization of subjects who were taking other

treatments for ALS if approved in their region of participation, specifically edaravone and sodium phenylbutyrate/taurursodiol if their treatment was considered stable at screening. In protocol amendment 4.0 the original riluzole stratification groups (on riluzole therapy vs. not on riluzole therapy) would be amalgamated, and randomization would be based on edaravone, sodium phenylbutyrate/taurursodiol, and neither, as indicated above.

After completion of the 24-week double-blind treatment phase, all subjects will be offered the opportunity to enter the optional 28 weeks long-term extension (LTE) Period of the study. Those who choose to enter the LTE Period will sign an additional informed consent form (ICF) and start open-label treatment with PTC857 250 mg BID. Those who choose not to enter the LTE Period will stop treatment, and a telephone follow-up visit will be conducted 4 weeks (±3 days) after the last dose of study treatment. At the end of the LTE Period, those who choose not to enter the Continued LTE Period will stop treatment, and a Telephone Follow-Up Visit will be conducted 4 weeks (±3 days) after the last dose of study treatment. Subjects who enter the Continued LTE Period (Part C) will continue treatment for an optional additional 108 weeks. All subjects will be treated with open-label PTC857 (250 mg BID) during the Continued LTE Period. Subjects will remain blinded to their original treatment from the Treatment Period (Part A). At the end of this period, a Telephone Follow-Up Visit will be conducted 4 weeks (±3 days) after the last dose of study treatment. Results from the Treatment Period (Part A), the LTE Period (Part B), and the Continued LTE Period (Part C) will be reported separately. The scheduled study visit assessments are presented in Section 11 (Appendix 1).

Total duration of the individual participation will be approximately 172 weeks, including a screening period of 8 weeks mandatory in duration, a double-blind treatment period of 24 weeks, a long-term open-label extension period of 28 weeks, a continued long-term extension period of 108 weeks, and a follow up period of 4 weeks. A subset of approximately 36 subjects from select sites participating in the PK sub-study will participate in serial blood sampling to characterize the PK of PTC857 in subjects with ALS. The study schema is shown below.

Figure 1: Study Design



1.2. Study Objectives

1.2.1. Long-Term Extension Period Objectives (Part B)

The objectives of the Long-Term Extension (LTE) Period of this study are to assess the following in subjects with ALS:

- Disease progression, survival, neuropsychological function, respiratory function, motor/limb and bulbar function, and effects on biomarker activity upon long-term treatment with PTC857
- Safety and tolerability upon long-term treatment with PTC857
- Quality of life upon long-term treatment with PTC857
- PK of PTC857

1.2.2. Continued Long-Term Extension Period Objectives (Part C)

- The objectives of the Continued LTE Period of the study are to assess the following in subjects with ALS:
- Safety and tolerability upon continued long-term treatment with PTC857
- Disease progression and survival upon continued long-term treatment with PTC857
- Quality of life upon continued long-term treatment with PTC857

1.3. Study Endpoints

1.3.1. Long-Term Extension Period Endpoints (Part B)

The LTE Period endpoints of this study are the following:

- Severity and number of TEAEs and TESAEs, change in clinical laboratory tests, physical examination, vital signs, and 12-lead ECGs during the LTE Period
- Change from baseline in ALSFRS-R after 52 weeks of treatment
- Change from baseline in slow vital capacity as assessed by PFTs after 52 weeks of treatment
- Change from baseline in motor/limb and bulbar function as assessed by the Modified Norris Scale after 52 weeks of treatment
- Change from baseline in neuropsychological function as assessed by the ALS CBS after 52 weeks of treatment
- Survival after 52 weeks of treatment
- Quality of life as assessed by ALSAQ-40 after 52 weeks of treatment
- Quality of life as assessed by EQ-5D-5L after 52 weeks of treatment
- Change from baseline in blood and urine biomarker activity after 52 weeks of treatment
- PK of PTC857

1.3.2. Continued Long-Term Extension Period Endpoints (Part C)

The Continued LTE Period endpoints of this study are the following:

- Severity and number of TEAEs and TESAEs during the Continued LTE Period

- Change from baseline in ALSFRS-R after 160 weeks of treatment
- Survival after 160 weeks of treatment
- Quality of life as assessed by ALSAQ-40 after 160 weeks of treatment

1.4. Randomization and Blinding

Subjects who satisfy all the inclusion and none of the exclusion criteria will be randomly assigned in a 2:1 ratio to receive one of the following two treatment arms during the 24-week treatment phase according to a study randomization scheme:

- Arm 1: PTC857 (250 mg) administered orally BID for 24 weeks.
- Arm 2: Matching placebo administered orally BID for 24 weeks.

Investigators, subjects and site staff will be blinded to treatment assignments during the entire study period. PTC biostatistics staff who are directly involved in the analysis of the study results and members of the clinical study team will remain blinded to the treatment assignment throughout the conduct of Part A and will be unblinded after the first database lock for Part A.

Participation in the long-term extension periods does not involve randomization.

2. ANALYSIS SETS

2.1. All Enrolled Set

The All-Enrolled set will contain all subjects who signed an informed consent for this study. This analysis set will be used to summarize subject disposition.

2.2. Intent-to-Treat (ITT) Analysis Sets

Intent-to-Treat 1 Analysis Set (ITT1)

All subjects who are randomized after the Screening Period and take at least 1 dose of double-blind study drug in the Treatment Period and who have a decrease in the ALSFRS-R score of ≤4 points during the Screening Period will be included in the ITT1 Analysis Set. Subjects will be analyzed according to their randomized treatment.

Intent-to-Treat 2 Analysis Set (ITT2)

All subjects who are randomized after the Screening Period and take at least 1 dose of double-blind study drug in the Treatment Period will be included in the ITT2 Analysis Set. Subjects will be analyzed according to their randomized treatment assignment. ITT2 analysis set will be used as supportive analysis population.

2.3. Safety Analysis Set

All subjects in the ITT2 Analysis Set who receive at least 1 dose of study drug will be included in the Safety Analysis Set. Subjects will be analyzed according to the actual treatment received.

2.4. Long-Term Extension Safety Analysis Set

All subjects who receive at least one dose of the open-label study drug during the long-term extension periods will be included in the LTE Safety Analysis Set.

2.5. Per Protocol Set

All subjects in the ITT1 Analysis Set who have no major protocol deviations that affect the validity of the efficacy measurements will be included in the PP Analysis Set. The PP Analysis Set will be used for sensitivity analysis of the efficacy endpoint.

2.6. Pharmacokinetic Analysis Set

All subjects in the ITT2 Analysis Set who have at least 1 measurable post-baseline plasma or CSF PTC857 concentration will be included in the PK Analysis Set. Subjects with protocol violations that could affect the PK parameters will be assessed by the pharmacokineticist for inclusion into the PK analysis set. The pharmacokineticist may also exclude subjects or specific concentrations from the PK parameter calculation if required, for example if doses were missed or if samples were collected out of the allowed assessment windows. PK summaries and analyses will be conducted using the PK analysis set. The PK analysis set will be based on the actual treatment received.

2.7. Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the subject, investigator, or site staff. Examples of deviations include, but are not limited to, the following:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc (either the tests were not done, the incorrect tests were done, or the tests were not done within the time frame specified in the protocol)
- Procedural deviations such as incorrect storage of study drug, failure to update the ICF when new risks become known, or failure to obtain IRB/IEC approvals for the protocol and ICF revisions

Major protocol deviations are defined in the study's Protocol Deviation Handling Plan and include quality issues that have a high likelihood to significantly impact a subject's rights, safety or well-being, or have a potentially significant impact on data integrity.

Protocol deviations will be documented separately in a stand-alone file before database lock which include deviation category (e.g., violation of inclusion and exclusion criteria at screening, use of excluded concomitant medications, received the wrong treatment or incorrect dose), deviation description, severity (minor/major), visit/time point for each deviation.

3. GENERAL CONSIDERATIONS

3.1. General Considerations

Continuous data will be summarized using number of observations (n), arithmetic mean (mean), standard deviation (SD), median, minimum value (min) and maximum value (max). Additionally, for biomarker data, the summaries will include geometric means and geometric standard deviations, as appropriate. Categorical variables will be summarized using the frequency count (n) and percentage (%).

For all percentage calculations, the denominator will be the number of subjects in the analysis set, unless otherwise stated.

Only data from protocol scheduled visits will be included in the summary tables. Data from unscheduled visits will not be included in the summary tables but will be included in the by-subject listings. Descriptive summaries by visit will be provided for all the primary and secondary efficacy endpoints.

3.2. Data Definitions and Analysis Issues

3.2.1. Study/Onset Day

The study/onset day will be defined as the number of days after the first dose of study drug, which is assigned as Day 1 for analysis purposes. Study/onset day is calculated using the formula below:

Assessment Date Relative to First Dose of Study Drug	Study Day Calculation
Assessment/Event date prior to date of first dose	Date of assessment – Date of first dose of study drug
Assessment/Event date on or after Date of first dose	(Date of assessment – Date of first dose of study drug) +1

3.2.2. Weeks Since Initial Dose of Investigational Product (PTC857)

Nominal visits for subjects initiating open-label PTC857 in Part B will be aligned with those who initiated PTC857 during the double-blind period (Part A).

This alignment allows for the evaluation of changes from initial exposure for scheduled assessments (e.g., laboratory tests, and vital signs) for subjects initiating PTC857 at different periods of the study, i.e., those randomized to active drug in Part A at the randomization visit (Day 1) and those randomized to placebo and initiating active drug in Part B. The display below illustrates the matching of these two groups.

Study Day LTE period	Study Day Double-blind period	Weeks since initial exposure to PTC857
Day 169	Day 1	0
Day 183	Day 14	2
Day 197	Day 29	4
Day 225	Day 57	8
Day 253	Day 85	12

Day 365	Day 113	28
Day 393	Day 141	32
Day 449	NA	40
Day 533	NA	52
Day 617	NA	64
Day 701	NA	76
Day 785	NA	88
Day 869	NA	100
Day 953	NA	112
Day 1037	NA	124
Day 1121	NA	136
Day 1149	NA	140

3.2.3. Study Baseline Definition and Change from Baseline

3.2.3.1. Baseline Definition and Change from Baseline

The Baseline Visit will be defined as Day 1 of the study. Unless otherwise stated, for each subject, baseline value of a parameter is defined as the last non-missing measurement taken at most one week prior to the first dose of study drug (including unscheduled assessments). If the last non-missing measurement and the date of first dose of study drug coincide, that measurement will be considered baseline, but AEs and medications commencing on the date of first dose of study drug will be considered post-baseline.

For the purposes of calculating summary statistics of the outcome measures, change from baseline will be derived as follows:

- Change from baseline = Post-baseline value – Baseline value

3.2.4. Duration of Event

Where the duration of an event is to be calculated, it will be derived as:

Duration (days) = (Event stop/end date – Event start date) + 1.

Duration (months) = Duration (days) / 30.4375

3.2.5. Retest and Unscheduled Visits

Unscheduled measurements and retests will not be included in by-visit summaries but will contribute to incidence of significant abnormality tables, where applicable.

In the case of a retest (same visit number assigned), the last available measurement for that visit will be used for by-visit summaries.

3.2.6. Study Treatment Periods

Period	Definition
Pre-treatment Period	Date of Screening through one day prior Date of first dose
Double-blind	Date of first dose through one day after Date of last dose

Treatment Period	OR Date of first dose through one day prior first dose during open-label treatment period
Open label Long-term Extension Periods	Date of first dose during open-label LTE period (Part B) to Date of last dose during open-label LTE period (Part B or C) plus one OR Date of first dose during open-label LTE period (Part B) through one after Date of last dose during open-label continued-LTE period (Part C)

3.2.7. Coding Dictionary

Where applicable, safety data will be coded using the following coding dictionaries:

Dictionary	Version
Medical Dictionary for Regulatory Activities (MedDRA)	25 or higher
World Health Organization Drug Dictionary (WHODrug Global)	WHODrug-Global-B3 Sep 2021 or later

3.2.8. ALS Functional Rating Scale - Revised (ALSFRS-R) score

The ALSFRS-R is a quickly administered (5-minute) ordinal rating scale that assesses the subjects' capability and independence in 12 functional activities across 4 subdomains of bodily function (bulbar, gross motor, fine motor, and respiratory) relevant in ALS. Each activity is recorded to the closest approximation from a list of 5 choices, scored 0 (total loss of function) to 4 (no loss of function), with the total score ranging from 0 to 48 and higher scores indicating less functional impairment (Cedarbaum 1999).

The score change from baseline will be calculated as the post baseline score minus the baseline score, so that a positive score for change from baseline would indicate improvement in function relative to baseline, while a negative score indicates worsened disease relative to baseline.

Subdomains	Items
Bulbar	Speech, Salivation and Swallowing
Fine Motor	Handwriting, Cutting Food/Handling Utensils and Dressing Hygiene
Gross Motor	Turning in Bed, Walking and Climbing Stairs
Respiratory	Dyspnea, Orthopnea and Respiratory Insufficiency

3.2.9. Pulmonary Function Tests

Pulmonary function tests are used to assess respiratory function. Pulmonary function tests (PFTs) will assess SVC and SNIP. Forced vital capacity, vital capacity, forced expiratory volume, and maximum inspiratory pressure will not be measured. Oxygen saturation (SpO2), respiratory rate, and heart rate will be assessed in both upright and supine positions. The SNIP test will assess nasal pressure in each unilaterally occluded nostril during a maximal sniff to assess inspiratory muscle strength.

3.2.10. Modified Norris Scale

Modified Norris scale is used to assess the motor/limb and bulbar function. The Modified Norris Scale is a rating scale for ALS that consists of 2 parts: the Limb Norris Scale and the Norris Bulbar Scale. The Limb Norris Scale has 21 items to evaluate extremity function, and the Norris Bulbar Scale has 13 items to evaluate bulbar function. Each item is rated in 4 ordinal categories. The total score is calculated by summing all the scales (Norris 1974).

3.2.11. ALS Cognitive Behavioral Screen

The ALS CBS is a brief measure of cognition and behavior in patients with ALS. The ALS CBS is composed of 2 sections: cognitive and behavioral. The cognitive section includes commonly used elements of standard testing batteries, consisting of 8 tasks that assess attention, concentration, tracking/monitoring, and initiation and retrieval. Each subtest score ranges from 0–5 and the total score ranges from 0–20. The behavioral section is composed of questions sensitive to organic brain changes. It consists of fifteen 3-point Likert items (total score ranges from 0–45) questioning caregivers on patients' behavioral changes (3 = "no change"; 0 = "large change") and 4 "yes/no" questions on anxiety and depression.

3.2.12. ALSAQ-40 Assessment

The ALSAQ-40 is a disease-specific measure of health-related quality of life for ALS (Jenkinson 1999). It is specifically used to measure the subjective well-being of patients with ALS and provides scores for 5 scales: physical mobility (10 items: 1-10), activities of daily living and independence (10 items: 11-20), eating and drinking (3 items: 21-23), communication (7 items: 24-30) and emotional reactions (10 items: 31-40). If one of the question items of ALSAQ-40 is missing, the total score will be considered as a missing value. However, it may be included in the summary of the specific scales. Lower total scores for the ALSAQ-40 indicate better quality of life, with higher scores indicating poorer quality of life. Analyses will be performed on the total score.

3.2.13. EuroQol EQ-5D-5L

The EQ-5D-5L is a patient reported outcome measure (PROM) that determines a subject's health-related quality of life (www.euroqol.org). EQ-5D-5L consists of 2 components: The health state descriptive system and the visual analogue scale (EQ-VAS). The health state descriptive system is a 5 Dimension (5-D) questionnaire covering 5 health related aspects: Mobility, self-care, usual activities, pain/discomfort and anxiety/depression. For each Dimension, the subject responds by selecting one of 5-levels (5L): 1=No problems; 2=Slight Problems; 3=Moderate Problems; 4=Severe Problems and 5=Extreme Problems.

The visual analogue scale (EQ-VAS) is a 20 cm vertical graduated visual analogue scale with numbers ranging from 0 (=worst imaginable health) to 100 (=best imaginable health). Subjects rate their overall health by placing an X mark on the scale to indicate their perceived overall health.

3.2.14. Survival

The survival endpoint is defined as the time from the randomization date to the date of death from any cause.

3.3. Interim Analysis

No formal interim analyses are planned for this study. However, At the time of the database lock of the double-blind treatment period (Part A) selected data from the open-label long term extension periods (combined Parts B and C) will be descriptively summarized and included in the interim clinical study report. This (select data) will include:

- All available safety endpoints described in Section 6
- All available survival data
- All available ALSFRS-R data
- All available ALSAQ-40 data

3.4. Changes to Protocol Specified Analysis

Safety data for Parts B and C will be combined, as the transition from Part B to Part C does not represent a clinically significant timepoint.

4. SUBJECTS DATA

4.1. Subject Disposition

Subject disposition data will be summarized. A summary table of subject disposition will be produced reflecting:

4.1.1. Subject Disposition – Part B

- Subjects completed in Part A
- Subjects enrolled (or consented) in part B
- Safety analysis set for Part B (Subjects received treatment in Part B)
- Subjects completed in Part B
- Subjects who early terminate the study treatment during part B and reasons for early termination
- Subjects who early terminate the study in Part B and reasons for early termination

4.1.2. Subject Disposition – Part C

- Subjects completed in Part A
- Subjects completed in Part B
- Subjects enrolled (or consented) in part C
- Subjects dosed in Part C
- Subjects completed in Part C
- Subjects who early terminate the study treatment during part C and reasons for early termination
- Subjects who early terminate the study in Part C and reasons for early termination

For the enrolled population in the LTE parts, the number and percentage of participants in each analysis population will be presented.

4.2. Demographics and Baseline Characteristics

Subjects' demographics and baseline characteristics will be summarized overall and by treatment group for subjects in the safety analysis sets for Part B and C.

Continuous variables (e.g., age and weight) will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). Qualitative variables (e.g., sex, race, and ethnicity) will be summarized with counts and percentages.

The demographic parameters will include age category (<65 and ≥65 years), gender, race, and ethnicity. Age will also be summarized as a continuous variable. The baseline parameters include, height, body weight, BMI, ALSFRS-R score, amount of change in ALSFRS-R score during the screening period (<1, 1-2, 3-4, or >4 points total loss) use of standard of care (SoC) medications for ALS at baseline and disease characteristics at screening.

By-subject listings of demographic and other baseline characteristics will be provided for all randomized subjects. In this listing flag variables will be used to identify subjects in various analysis sets.

4.3. Disease Characteristics

Subjects' disease characteristics will be summarized for subjects in the Long-Term Extension Safety Analysis. By-subject listings of disease characteristics will also be provided. Duration of symptoms (months) and months since diagnosis will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). The following disease characteristics will be summarized with counts and percentages (n (%)):

- Site of symptom onset
- Family history
- El Escorial criteria at screening
- Pre-ALS impairment
- Months since ALS symptom onset
- Months since diagnosis
- Site of onset
- Diagnosis delay
- Derived King's Clinical Stage (Balendra 2014 also see Appendix 5)
- TRICALS Risk Profile

The TRICALS risk profile (van Eijk 2021) is based on the European-Network-to-Cure-ALS survival prediction model, a cross-validated model to predict the composite endpoint death or respiratory insufficiency based on data of 11,475 subjects with ALS (Westeneng 2018). The TRICALS Risk Profile is a weighted average of seven pre-randomization subject characteristics, including: Percent predicted vital capacity, age at onset in years, diagnostic delay (months from symptom onset to diagnosis), presence of frontotemporal dementia, progression rate of the ALS functional rating scale (points per month), Bulbar onset of ALS, and an El Escorial criteria of clinically definite ALS at screening. The range of possible values for this summary predictor is from approximately -12.0 to 0.0 with higher scores indicating a poorer prognosis. Section 13 (Appendix 3) provides details on its derivation.

4.4. Medical History

Medical and surgical history will be provided for subjects in the long-term extension safety set sets for Part B and C. Medical history will also be provided for all subjects who were exposed to the investigational product (PTC857).

Medical and surgical history will be coded using a central coding dictionary, the Medical Dictionary for Regulatory Activities (MedDRA) terminology.

Medical and surgical history will be defined as any significant past medical conditions that ended before screening or current medical conditions that were ongoing at Screening. Medical and surgical histories will be summarized separately by system organ class (SOC) and preferred term

(PT) overall and for each treatment group for the safety analysis set. Summaries will be sorted alphabetically by SOC, and by descending order of frequency for PTs according to the total column. Subjects with more than one of the same PTs within an SOC will be counted only once. A by-subject listing will be provided for the safety analysis set.

4.5. Prior Medications and Concomitant Medications

Prior medications are medications which started and stopped before the first dose of study drug. Any changes to prior medications (dose, regimen, etc.) during the study will be considered a new concomitant medication. In the analyses of subjects exposed to the investigational product (PTC857) prior medications will be defined as medications started and stopped before the first dose of PTC857.

Concomitant' medications/procedure are medications/procedures which started prior to or after the first dose of study drug and ended after the date of first dose of study drug or ongoing at the end of the study. If a medication or procedure cannot be classified as "prior" or "concomitant" after applying imputation rules for missing/incomplete dates, it will be classified as concomitant.

Prior and concomitant medications will be coded using the latest available version of the Anatomical Therapeutic Chemical (ATC) classification text from the World Health Organization Drug Dictionary (WHO DD) and summarized separately. The number and percentage of subjects taking a medication will be displayed by therapeutic class (ATC Level 3) and preferred name. Summaries will be sorted alphabetically by ATC-Level 3, and by descending order of frequency for preferred name according to the total column. Subjects with more than one of the same preferred names within an ATC-Level 3 will be counted only once. Prior medications and concomitant medications will be summarized for subjects in the LTE safety analysis set and for the subjects exposed to PTC857.

Prior and concomitant procedures will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology.

The count and percentage of subjects who have undergone various prior and concomitant procedures will be summarized by treatment received.

A by-subject listing of prior and concomitant medications will be provided for the safety analysis set.

4.6. Multiplicity

There will be no multiplicity consideration.

4.7. Missing Data

For the analysis of change from baseline in ALSFRS-R at weeks 52 and 160, missing outcome data will be assumed to be missing at random (MAR) and will be imputed implicitly using a mixed model repeated measures analysis.

4.8. Treatment Compliance

Percent compliance will be calculated as the amount of dose taken divided by the amount of dose that should have been taken multiplied by 100. The following formula will be used to calculate compliance.

$$Compliance\ (\%) = \frac{100 \times (2 \times treatment\ duration\ in\ days - number\ of\ missed\ doses)}{2 \times treatment\ duration\ in\ days}$$

The extent of exposure to study medication (treatment duration) in days will be calculated as the last dose date minus the first dose date + 1.

Exposure (number of days) and compliance will be summarized descriptively (number of doses taken / number of doses expected and percentage) for the LTE safety analysis set and for subjects exposed to PTC857.

4.9. Visit Windows for The Early Termination Visit and Unscheduled Visits

If a subject completely withdraws from the study prematurely, and unless consent is withdrawn, all efforts are made to collect efficacy and safety data and to complete the Early Termination Visit (ET).

Follow-up efficacy data collected at the early termination visit will be incorporated into the study visit schedule using the visit windows, defined in Table **1**, Table **2** and Table 3. Similarly, follow-up efficacy data collected during unscheduled visits or make-up visits will also use the same visit windows. If a follow-up visit (ET) or unscheduled visit falls in the same visit window as a scheduled visit, then the scheduled visit data will be used. If an unscheduled visit and ET visit fall within the same window, then the visit closest to the target day will be used.

Table 1: Visit Windows for The Early Termination and Unscheduled Visits

	ALSFRS-R		
Visit	Lower Bound	Target Day	Upper Bound
Day 29	15	29	42
Day 57	43	57	70
Day 85	71	85	98
Day 113	99	113	126
Day 141	127	141	154
Day 169	155	169	182
Day 197	183	197	210
Day 225	211	225	238
Day 365	351	365	378
Day 449	435	449	462
Day 533	519	533	546
Day 617	603	617	630
Day 701	687	701	714
Day 785	771	785	798
Day 869	855	869	882
Day 953	939	953	966
Day 1037	1023	1037	1050
Day 1121	1107	1121	1134

Table 2: Visit Windows for The Early Termination and Unscheduled Visits

	SVC, Norris Scale, and plasma NfL		
Visit	Lower Bound	Target Day	Upper Bound
Day 85	64	85	106
Day 169	148	169	190
Day 253	232	253	274
Day 365	344	365	386

Table 3: Visit Windows for The Early Termination and Unscheduled Visits

	ALSAQ-40		
Visit	Lower Bound	Target Day	Upper Bound
Day 85	64	85	106
Day 169	148	169	190
Day 253	232	253	274
Day 365	344	365	386
Day 449	428	449	470
Day 533	512	533	554
Day 617	596	617	638
Day 701	680	701	722
Day 785	764	785	806
Day 869	848	869	890
Day 953	932	953	974
Day 1037	1016	1037	1058
Day 1121	1100	1121	1142

5. EFFICACY ANALYSIS

5.1. Primary Efficacy Analysis

Not applicable

5.2. Analysis of Efficacy Endpoints in the LTE Period

Analyses of the LTE efficacy endpoints will compare subjects randomized to PTC857 (early start of PTC857) against those randomized to Placebo (late start of PTC857). The two treatment groups will be labelled as utreloxastat/utreloxastat and Placebo/utreloxastat and will include all available efficacy results from the entire study period (parts A, B and C).

Analyses will be performed for both the ITT1 and ITT2 analyses sets.

5.2.1. Change from Baseline in ALSFRS-R

Change from baseline in ALSFRS-R at Weeks 52 and 160 will be evaluated by means of a mixed model repeated measures analysis using all available ALSFRS-R scores up to Week 160.

The model will include fixed categorical effects of treatment (PTC857 or placebo), use of approved background therapy for ALS at screening (Yes vs No), the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2 or 3-4 points total loss), time of collection (nominal week at the visit as a categorical variable), as well as the continuous, fixed covariates of baseline ALSFRS-R, and The TRICALS risk profile. In addition, the following interaction terms will be included: Time by treatment, time by baseline ALSFRS-R, and TRICALS risk profile by time. Subject will be included as a random effect. The Restricted Maximum Likelihood (REML) estimation approach will be used with the variance-covariance structure set as unstructured (UN). Other types of covariance matrices will be explored in the case of non-convergence of the UN. Subjects with no post baseline results for the outcome variable will be excluded. This analysis will be performed for both the ITT1 and ITT2 analysis sets.

Observed and change-from-baseline values will also be summarized descriptively at each visit.

In supplementary analyses, score changes from baseline at Weeks 52 and 160 for the ALSFRS-R subdomains will be analyzed under the same estimand framework where MMRM models will be used to implicitly impute missing data. Nominal P-values for the treatment arm comparison, estimate of LSMEANS for the treatment differences (PTC857 – Placebo) at Weeks 52 and 160, and the two-sided 95% confidence intervals of the LSMEANS difference will be presented.

To determine if there is a reduction in the decline of ALSFRS-R scores in subjects randomized to placebo after starting open-label PTC857, graphical plots comparing the decline during the double-blind period and the open-label extension periods will be provided. Additionally, changes (in ALSFRS-R) from both the initial study baseline and the treatment baseline in Part B will be reported.

5.3. Analysis of Continuous Long Term Extension Endpoints

The following continuous long term extension endpoints will be analyzed for both the ITT1 and ITT2 analysis sets using a similar model as described in Section 5.2.1, p-values for the treatment arm comparison, estimate of LSMEANS for the treatment differences (PTC857 – Placebo), and the two-sided 95% confidence intervals of the LSMEANS difference will be presented:

- Quality of life as assessed by Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) at 52 and 160 weeks.
- Change from baseline in Modified Norris Scale total score at 52 weeks.
- Change from baseline in slow vital capacity (SVC) as assessed by pulmonary function tests (PFTs) at 52 weeks.
- Change from baseline in neuropsychological function as assessed by the ALS CBS at 52 weeks.
- Change from baseline in log transformed NfL (plasma) at 52 weeks. Geometric means and mean ratios to baseline, as well as the difference in geometric mean ratio for those in the PTC875 arm to those in the Placebo arm will be reported, together with 95% confidence intervals and p-values.

5.4. Overall Survival

Overall survival (OS) is defined as the time in months from the date of first dose to the date of death from any cause or date last known alive for those who did not die.

Specifically,

OS = Date of death or date last known alive – Date of first dose + 1

Subjects will be censored at the last date they are known to be alive. The last known alive date will be the last date of any subject record in the study database. The date may be the last visit date or last contact date that the subject is known to be alive. Subjects who only have baseline record will be censored at the first dose date.

OS will be analyzed using KM methods. A summary table of the number of deaths, the number of censors, and KM estimates (25th, 50th, 75th percentile of OS along with the 95% CI). Kaplan-Meier survival curves will also be presented.

A stratified Cox Proportional Hazards regression model will be used to estimate the hazard ratio (and 95% CIs) of PTC857 compared to Placebo. The model will be stratified by the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2 and 3-4-points total loss), and use of approved background therapy for ALS at screening (Yes vs No). In addition, the TRICALS risk profile and ALSFRS-R score at baseline will be included in the model as covariates. Ties in survival time will be handled using the Breslow method.

Kaplan-Meier survival curves will also be presented.

5.5. Analysis of Exploratory Endpoints in the LTE

Exploratory endpoints will be summarized for both the ITT1 and ITT2 analysis sets. Summary statistics by nominal timepoint will be provided for all the exploratory endpoints, including ALS-CBS, EQ5D-5L, and urine p75 neurotrophin receptor (p75NTR).
ALS-CBS total score will be analyzed using the same methodology described for secondary (continuous) endpoints in Section 5.3. P-values for the treatment arm comparison at week 52, estimate of LSMEANS for the treatment differences (PTC857 – Placebo), and the two-sided 95% confidence intervals of the LSMEANS difference will be presented.

6. SAFETY ANALYSES

Safety will be assessed based on exposure, and will include adverse events, laboratory assessments, vital signs, 12-Lead electrocardiogram, physical examination, and Columbia-Suicide Severity Rating Scale (C-SSRS). The results will be descriptive in nature. All data will be summarized and listed. There will be no statistical comparisons between the treatment groups for safety data, unless otherwise specified within the relevant section. All data will be summarized and listed.

6.1.1. Pooling of Safety Analyses

Safety data will be reported for the pooled open-label long-term extension period (Parts B and C) as well as for the entire pooled study period (Parts A, B, and C).

Safety summaries for the open long-term extension periods, (Parts B and C) will be based on The Long-Term Extension Safety Population (defined in Section 2.4). These summaries will be displayed by actual treatment received during the double-blind treatment period and long-term extension periods. Tabular outputs will have columns labeled as follows:

- Placebo / Utreloxastat (subjects initially randomized to placebo)
- Utreloxastat / Utreloxastat (subjects initially randomized to utreloxastat)
- Total

The safety summaries for the entire pooled study period (Parts A, B, and C) will be presented in a single total column and will be limited to the subset of the Safety Analysis Set consisting of subjects who received the actual investigational product (PTC857).

6.2. Extent of Exposure

The extent of exposure to study drug will be summarized descriptively. The summaries will include mean, SD, median, minimum, and maximum values.

The extent of study drug exposure for the open label long-term extension period is defined as the difference between the last dose of study medication and the first dose study drug in the open label long-term extension period plus 1.

Extent of exposure to PTC857 during the whole study period will also be summarized. Extent of exposure to PTC857 will be defined as the difference in time between first exposure to PTC857 and the last dose of PTC857.

6.3. Adverse Events

6.3.1. Reporting of Adverse Event Data

Adverse events will be collected and recorded from the time subjects sign the informed consent form to the end of the follow-up period. All AEs will be classified by primary system organ class (SOC) and preferred term (PT) according to the MedDRA coding dictionary. A treatment-emergent adverse event (TEAE) is an AE that begins after the first administration of study drug or any existing AEs that worsens after the first dose of study drug. All reported AEs will be listed, but only TEAEs will be summarized in tables.

AEs with an onset from day 1 of the open label long-term extension periods up to and including 30 days after the last dose of study drug in the open label long-term treatment periods (or up to and not including the start date of the Continued LTE period whichever comes first) will be considered as occurring during the LTE treatment period.

An overall summary of TEAEs, including the number and percentage of subjects reporting at least one TEAE for the categories listed below, will be provided.

- Subjects with at least one TEAE
- Subjects with at least one TEAE related to study drug
- Subjects with at least one serious TEAE
- Subjects with at least one non-serious TEAE
- Subjects with at least one serious TEAE related to study drug
- Subjects with at least one TEAE leading to premature discontinuation of study drug
- Subjects with at least one TEAE leading to death
- TEAEs by highest relationship to study drug
- TEAEs by highest severity

The following summaries will be tabulated:

- TEAEs overall and by SOC and PT
- TEAEs occurring in ≥ 5% of subjects within a treatment group, by PT
- TEAEs by PT
- Study drug related TEAEs, overall and by SOC and PT
- TEAEs by maximum severity, overall and by SOC and PT
- Grade 3 or Higher TEAEs by SOC and PT
- Grade 3 or higher related TEAE by SOC and PT
- TEAEs by highest relationship to study drug
- Serious TEAEs, overall and by SOC and PT
- Serious TEAEs, overall and by severity grade, SOC and PT
- Non-serious TEAEs, overall and by severity grade, SOC and PT
- TEAEs leading to discontinuation of study drug, overall and by SOC and PT
- TEAEs leading to death, overall and by SOC and PT

TEAEs with “possibly related”, “probably related” or “related” to study drug based on the investigator assessment will be considered as drug related TEAEs. TEAEs with missing causality, if any, will be assumed as ‘Related AEs’.

For summaries by SOC and PT, a subject will be counted only once at the SOC level and once at each PT within the SOC level, even if the subject experienced more than one AE within a

specific SOC and PT. For summaries by SOC, PT, and maximum severity, a subject will be counted only once at the maximum severity level for which the event occurred at the SOC level and at the maximum severity level for which the event occurred for each unique PT within that SOC level. Therefore, subjects will contribute to only one severity level within a PT or SOC.

Summaries presenting frequency of TEAEs by SOC and PT will be ordered by overall descending frequency of SOC and then, within a SOC, by overall descending frequency of PT. Summaries presenting TEAEs by PT only will be ordered by overall descending frequency of PT.

TEAE summaries for the LTE periods (Parts B and C) will be displayed by actual treatment received during the double-blind period and the long-term extension periods.

TEAE summaries for the entire trial period (Parts A, B and C combined) will be presented with a total column only and will include subjects exposed to actual PTC857.

Subject listings of all adverse events will be provided. Deaths and other serious or clinically significant non-fatal adverse events will be listed separately.

6.3.2. Exposure Adjusted incidence rates (EAIR)

Exposure-adjusted incidence rates (EAIRs) of treatment-emergent adverse events (TEAEs) will be presented by treatment group, MedDRA System Organ Class (SOC), and preferred term, as well as by treatment group and MedDRA preferred term.

For each grouping of subjects (e.g., “All subjects” and “subjects on PTC857), the EAIR of a specific treatment-emergent event is defined as the number of subjects experiencing the event at least once, divided by the total exposure-adjusted follow-up time in person-years.

For subjects who experience the event, the exposure-adjusted follow-up time will be calculated from the first exposure (to PTC857 in Part B) until the first occurrence of the event. For subjects who do not experience the event (censored), the follow-up time will be calculated from the first dose during the safety reporting period until their last safety follow-up for that period.

EAIR will also be calculated for the subset of subjects exposed to the IP (PTC857), for those summaries, the start period of exposure will be defined at the date the subject first took PTC857.

Missing or partial AE onset dates will be imputed using the imputation rules described in Appendix 2.

6.4. Laboratory Values

All clinical laboratory analyses will be performed by the central laboratory. The clinical laboratory assessment of hematology, clinical chemistry, and urinalysis will be obtained at scheduled visits. The following parameters are collected at the following visits per protocol:

Parameter(s)	Scheduled Visit(s)
Hematology: Hemoglobin, Hematocrit, RBC count, Reticulocytes, Platelet count, WBC count, Neutrophils, Eosinophils, Monocytes, Basophils, Lymphocytes, Mean corpuscular volume	Screening, Day 1, Day 29, Day 57, Day 85, Day 169, Day 197, Day 225, Day 253, Day 365, Day 533, Day 701, Day 869, Day 1037, and ET
Clinical Chemistry: Urea, Creatinine, Fasting glucose, Sodium, Potassium, ALT, AST, GGT, Bilirubin (total, direct/indirect), Alkaline phosphatase, Albumin,	Screening, Day 1, Day 29, Day 57, Day 85, Day 169, Day 197, Day 225, Day 253,

Parameter(s)	Scheduled Visit(s)
	Day 365, Day 533, Day 701, Day 869, Day 1037, and ET
Lipid Profiles: Cholesterol (total), LDL, HDL, Triglycerides, LDH	Screening, Day 1, Day 14, Day 29, Day 57, Day 85, Day 169, Day 183, Day 197, Day 225, Day 253, Day 365, and ET
Urinalysis: Urobilinogen, Nitrite, pH, Glucose, Total protein, Erythrocytes, Leukocytes, Ketones, Microscopy, Specific gravity, Bilirubin	Screening, Day 1, Day 85, Day 169, , Day 253, Day 365, Day 533, Day 701, Day 869, Day 1037 and ET
Coagulation: PT [sec], PT [INR], aPTT	Screening, Day 1, Day 29, Day 57, Day 85, Day 169, Day 197, Day 225, Day 253, Day 365, Day 533, Day 701, Day 869, Day 1037, and ET
Pregnancy Test: β-HCG, FSH	Screening, Day 1, Day 29, Day 57, Day 85, Day113, Day141, Day 169, Day 197, Day 225, Day 253, Day 281, Day 309, Day 337, Day 365, Days 393, 421, 449, 477, 505, 533, 561, 589, 617, 645, 673, 701, 729, 757, 785, 813, 841, 869, 897, 925, 953, 981, 1009, 1037, 1065, 1093, and 1121 and ET

Separate summary tables and listings will be produced for each of the separate groups above (e.g., separate table for hematology and clinical chemistry etc.); however, urinalysis, and serum and urine pregnancy tests will be listed only. The International System of units (SI units) will be used for all summary and data listing displays unless otherwise indicated.

Laboratory data will be summarized descriptively by visit for each laboratory parameter. Both observed results and changes from baseline will be summarized for continuous variables. Laboratory values will be categorized as clinical grade (low/normal/high) according to normal ranges. Shifts from baseline to the last on-treatment value and the worst on-treatment value will be presented. For these shift summaries, for the last on-treatment summary only scheduled visits will be taken; for the worst on-treatment summaries unscheduled assessments will be included in the assessment of worst. Both worst low and worst high will be summarized.

Laboratory values that are below the limit of quantification will be imputed as the lower-limit of quantification, similarly, values above the limit of quantification will be imputed as the upper-limit of quantification for the summary statistics. But for listings, the values will be presented as recorded.

By-subject listings of all the laboratory parameters will be presented that clearly indicate out of normal range values and possible abnormal values for the safety analysis set.

6.5. Vital Signs

Vital signs including systolic and diastolic blood pressure (mmHg), pulse rate (beats/min), body temperature (ºC), Oxygen saturation (SpO2), and respiratory rate (breaths/min) will be evaluated at Screening, Day 1, Day29, Day57, Day 85, Day 113, Day 141, Day 169, Day 197, Day 225, Day 253, Day 365, Day 533, Day 701, Day 869, and Day 1037 as well as the ET visit where applicable.

For convenience, the conversion for temperature: Temperature (in °C) = (Temperature [in °F]-32) x (5/9).

Absolute results and changes from baseline will be summarized descriptively by visit for each parameter, by treatment group and overall.

6.6. 12-Lead Electrocardiogram

The following 12-lead ECG will be performed at Screening, Day 1, Day 85, Day 169, Day 253, Day 365, Day 533, Day 701, Day 869, and Day 1037 as well as the ET visit where applicable.

- Heart rate (bpm)
- QRS Interval (msec)
- PR Interval (msec)
- QT interval (msec)
- QTcF Interval (msec)
- Overall Interpretation

Descriptive summaries will be presented for the ECG parameter values and change from baseline by visit for all quantitative measurements. Overall interpretation of ECG assessment will be summarized by counts and percentages. QT and QTcF will be categorized as follows, and the category based on actual value will be summarized by treatment group at each visit as well as the category at each post-baseline visit based on change from baseline per the following criteria:

Type of Value	Category 1	Category 2	Category 3
Actual value	> 450 ms	> 480 ms	>500 ms
Change from Baseline	> 30 ms	>60 ms	

A by-subject listing of all ECG parameters will be provided.

6.7. Physical Examination

A detailed physical examination will be performed at Screening visit and targeted physical examination will be performed at Day 1, Day 85, Day 169, Day 253, Day 365, Day 533, Day 701, Day 869, and Day 1037 as well as the ET visit where applicable.

For the remaining visits, a symptom-driven physical examination will be performed as per the investigators' discretion. Physical examination including details of clinically significant findings, will be listed.

6.8. Columbia-Suicide Severity Rating Scale

The C-SSRS is a questionnaire that prospectively assesses suicidal ideation and suicidal behavior (Posner 2011). The C-SSRS, which uses a semi-structured interview to probe subject responses, is administered by an individual who has received training and certification in its administration. During the Screening Visit (Day -56), the "baseline" version of the C-SSRS is administered. This version assesses suicidal ideation and suicidal behavior during the subject's lifetime. At all subsequent visits, the "since last visit" version is administered.

Counts and percentage of subjects who experience suicidal ideation, suicidal behavior or neither (no ideation or behavior) will be summarized during the long-term extension periods and entire study period.

The C-SSR instrument consists of 11 Yes/No questions as shown in the table below and is grouped by question category, i.e., suicidal ideation questions and suicidal behavior questions. Within each type/group the questions are re-ordered by increasing severity. In addition, subjects are assessed for any self-injurious behavior without suicidal intent.

C-SSRS Item	Derivation
Suicidal ideation	A "yes" answer to any one of the following five questions. 1. Wish to be dead. 2. Non-specific active suicidal thoughts. 3. Active suicidal ideation with any methods (not plan) without intent to act. 4. Active suicidal ideation with some intent to act, without specific plan. 5. Active suicidal ideation with specific plan and intent.
Suicidal behavior	A "yes" answer to any one of the following five questions. • Preparatory acts or behavior • Aborted attempt • Interrupted attempt • Actual attempt • Suicidal behavior • Completed suicide
Self-injurious behavior without suicidal intent	A "yes" answer to the following question from suicidal behavior section on the C-SSRS: Has subject engaged in Non-Suicidal Self-Injurious behavior?
Suicidal Ideation Score	Is defined as the maximum suicidal ideation category (1-5 on the C-SSRS) present at the assessment. A score of 0 is assigned if no ideation is present.

For the overall assessment of suicidal ideation and behavior during the long-term extension periods a subject's worst finding during that period will be used.

The following tables will be generated at the end of the Continued LTE Period:

- Counts and Percentage of Subjects with Suicidal Ideation, Suicidal Behavior, and Self-Injurious Behavior without Suicidal Intent Based on the C-SSRS by Treatment group during the double-blind treatment and LTE period.
- Counts and Percentage of Subjects with Suicide-Related Treatment Emergent Events Based on C-SSRS by Treatment Condition during the Double-Blind Treatment Period and LTE period.
- Shift table in changes in C-SSRS categories from baseline to Week 160.
- Shift table in Changes in C-SSRS Suicidal Ideation scores from baseline to Week 160 by Treatment Condition.

All C-SSRS assessments will be listed.

6.9. Body Weight, BMI, Breast Imaging and Pregnancies

Bodyweight and BMI

Bodyweight is assessed at Screening, Day 1, Day 85, Day 169, Day 253, Day 365, Day 533, Day 701, Day 869 and Day 1037 as well as the ET visit where applicable. Height is assessed once at the screening visit.

The observed bodyweight and BMI values at each visit, along with the change from baseline to each post-baseline visit will be summarized descriptively as continuous variables by treatment group and overall. The following abnormality categorizations will additionally be summarized for body weight at each visit:

Category	Change from Baseline
Category 1	≥ 10% loss
Category 2	≥ 5 - 10% loss
Category 3	-5% < Weight change <+5%
Category 4	>5 - 10% increase
Category 5	≥ 10% increase

Breast Imaging and Pregnancies

At the investigator's discretion, an ultrasound or MRI may be performed for breast imaging in female subjects if clinically indicated. These examinations will take place on study days 169, 365, 701, and 1121.

Summary statistics will be used to describe the breast imaging results.

Pregnancy test results will be listed.

6.10. Subgroup Analyses of Safety

The count and percentage of subjects who report at least one TEAE will be summarized by treatment group and PT for the subgroups outlined below.

- Sex (male, female)
- Race (White, Non-white)
- Age at baseline (<65, ≥ 65 years)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino)
- Geographic region (North America, Europe, South America, Asia)
- Slow vital capacity (80%, >80%)
- Use of approved background therapy for ALS (Y/N)

7. PHARMACOKINETIC OUTCOMES

The PK analysis plan will be detailed in a separate PK analysis plan.

8. REFERENCES

Group, WGEM-AS. Safety and efficacy of edaravone in well-defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2017; 16(7): 505-512.

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 Apr;14(3):162-8. doi: 10.3109/21678421.2012.762930. Epub 2013 Jan 17. PMID: 23323713.

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, Nakanishi A. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999 Oct 31;169(1-2):13-21. doi: 10.1016/s0022-510x(99)00210-5. PMID: 10540002.

Norris FH, Calanchini PR, Fallat RJ, Panchari S, and Jewett B. The administration of guanidine in amyotrophic lateral sclerosis. Neurology, Vol 24 | Number 8, August 01, 1974.

Jenkinson C, Fitzpatrick R, Brennan C, Bromberg M, Swash M. Development and validation of a short measure of health status for individuals with amyotrophic lateral sclerosis/motor neurone disease: the ALSAQ-40. J Neurol. 1999 Nov;246 Suppl 3:III16-21. doi: 10.1007/BF03161085. PMID: 10631656.

Garcia-Hernandex, A and Rizopoulos, D. %JM: A SAS Macro to Fit Jointly Generalized Mixed Models for Longitudinal Data and Time-to-Event Responses. Journal of Statistical Software. 2018 Apr; 84(12), doi:10.18637/jss.v084.i12.

Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17:423-433

van Eijk RPA, Westeneng HJ, Nikolakopoulos S, et al. Refining eligibility criteria for amyotrophic lateral sclerosis clinical trials. Neurology 2019;92:451-460

van Eijk RPA, Roes KCB, van den Berg LH, Lu Y. Joint modeling of endpoints can be used to answer various research questions in randomized clinical trials. J Clin Epidemiol. 2022 Jul;147:32-39. doi: 10.1016/j.jclinepi.2022.03.009. Epub 2022 Mar 26. PMID: 35346783.

TRICALS Eligibility, Standard Operating Procedure, version 1.0, 2020, https://tricals.shinyapps.io/risk-profile/

van Eijk RPA, et al. Innovating Clinical Trials for Amyotrophic Lateral Sclerosis. Challenging the Established Order, Neurology Sep 2021, 97 (11) 528-536; DOI: 10.1212/WNL.0000000000012545

Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17:423-433

Rubika Balendra, Ashley Jones, Naheed Jivraj, Catherine Knights, Catherine M. Ellis, Rachel Burman, Martin R. Turner, P. Nigel Leigh, Christopher E. Shaw & Ammar Al-

Chalabi (2014) Estimating clinical stage of amyotrophic lateral sclerosis from the ALS Functional Rating Scale, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 15:3-4, 279-284, DOI: 10.3109/21678421.2014.897357

Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, Currier GW, Melvin GA, Greenhill L, Shen S, Mann JJ. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry. 2011 Dec;168(12):1266-77. doi: 10.1176/appi.ajp.2011.10111704. PMID: 22193671; PMCID: PMC3893686.

9. PROGRAMMING CONSIDERATIONS

All tables, figures, listings (TFLs), and statistical analyses will be generated using SAS for Windows, Release 9.4 or higher (SAS Institute Inc., Cary, NC, USA). Computer-generated table, listing and figure output will adhere to the following specifications.

1. One SAS program can create several outputs.
2. Each output will be stored in a separate file.
3. Output files will be delivered in Word format.
4. The numbering of TFLs will follow ICH E3 guidance.
5. All tables and listings will be produced in landscape orientation. Figures use the orientation that best facilitates interpretation of the data.
6. All TFLs will be produced using the Courier New font, size 10. The smallest acceptable point size for the Regulatory Authorities is 8.
7. Headers and footers for figures will be in Courier New font, size 8, which is the smallest acceptable point size for the Regulatory Authorities.
8. TFLs will be in black and white (no color), unless otherwise specified.
9. Specialized text styles, such as bolding, italics, borders, shading, and color are not used in the tables and listings, unless otherwise specified. Color may be used in figures containing multiple plotted lines as long as information is not lost when the figure is reproduced in monotone.
10. A mixed case will be used for titles, footnotes, column headers, and programmer-supplied formats, as appropriate.
11. For categorical parameters, all categories will be presented in the table, even if n=0 for all treatment groups in each category. For example, the frequency distribution for symptom severity would appear as:

Severity Rating	n
Mild	2
Moderate	5
Severe	0

- An Unknown or Missing category will be added to each parameter for which information is not available for 1 or more subjects.
- Unless otherwise specified, the estimated mean, median, Q1 and Q3 for a set of values will be printed out to 1 more significant digit than the original values, and standard deviations and standard errors will be printed out to 2 more significant digits than the original values. The minimum and maximum will report the same significant digits as the original values.
- Confidence intervals are displayed within round brackets and separated by a comma. The lower limit is presented first, and the upper limit is presented second. The confidence interval is placed either beside or below the corresponding estimate. The estimate and the confidence limits are presented using the same degree of precision.

- P-values will be output in the format: '0.xxx', where xxx is the value rounded to 3 decimal places. Every p-value less than 0.001 will be presented as <0.001. If the p-value is returned as >0.999, then present as >0.999.
- Percentage values will be printed to one decimal place. Unless otherwise noted, for all percentages, the number of subjects in the analysis population for the treatment group who have an observation will be the denominator. Percentages after zero counts will not be displayed and percentages equating to 100% will be presented as 100%, without decimal places.
- Tabular display of data for medical history, prior/concomitant medications, and all tabular displays of adverse event data will be presented by the body system, treatment class, or SOC with the highest occurrence in the active treatment group in decreasing order, assuming all terms are coded. Within the body system, drug class and SOC, medical history (by preferred term), drugs (Medication class), and adverse events (by preferred term) will be displayed in decreasing order. If incidence for more than 1 term is identical, they will then be sorted alphabetically.
- Missing descriptive statistics or p-values which cannot be estimated will be reported as '-' Missing values for numeric and character variables are displayed as blanks in data listings.
- The percentage of subjects will normally be calculated as a proportion of the number of subjects assessed in the relevant treatment group (or overall) for the analysis population presented. However, careful consideration is required in many instances due to the complicated nature of selecting the denominator, usually the appropriate number of subjects exposed. Details will be described in footnotes or programming notes, as necessary.
- If no relevant data are available at all for a display, then present "No data to Report".

10. MOCK TABLES, LISTINGS, AND GRAPHS

The study tables, listings and graphs shells will be provided in a separate document.

11. APPENDIX 1: SCHEDULE OF ASSESSMENT

Schedule of Assessments for Screening and Double-Blind Treatment Period (Part A)

			Treatment Period (24 W)								EOS/ FU	Early Termination
Study Day	**Screening (-56 Days) (±5)**	**Day -28 (±3)**	**Day 1 (-3)[a]**	**Day 14 (±3)**	**Day 29 (±3)**	**Day 57 (±3)**	**Day 85 (±3)**	**Day 113 (±3)**	**Day 141 (±3)**	**Day 169 (±3)**	**Day 197 (±3)**	
			Baseline[a]							**End of Treatment[b]**	**Follow-Up[c]**	**ET[d]**
Telephone call/in-home or in-person visit[f]	IP	PC	IP	PC/IH	PC/IH[e,f]	PC/IH[f]	IP	PC/IH[f]	PC/IH[f]	IP	PC	IP
COVID-19 PCR	X											
Informed consent	X						X[g]			X[h]		
Inclusion/exclusion criteria	X											
Re-evaluation eligibility			X									
Randomization			X									
Height	X											
Weight and BMI	X		X				X			X		X
Demographics	X											
Medical history	X											
Serum/urine pregnancy test (females only)[j]	X		X		X	X	X	X	X	X	X	X
FSH measurement[j]	X											
12-Lead ECG[k]	X		X				X			X		X
Vital signs[l]	X		X		X	X	X	X	X	X		X
Physical exam[m]	X		X				X			X		X
Sparse PK blood sampling[n]			X				X			X		X
Clinical laboratory tests[o]	X		X	X[p]	X	X	X			X		X
CSF for biomarkers[q,r]			X							X		X
Laboratory biomarkers (blood and urine)[s]			X				X			X		X
ALSFRS-R[t]	X	X	X		X	X	X	X	X	X	X	X
PFTs[u]	X		X				X			X		X
ALS CBS	X		X				X			X		X
Modified Norris Scale			X				X			X		X
ALSAQ-40			X				X			X		X
EQ-5D-5L			X				X			X		X
C-SSRS[v]	X	X	X		X	X	X	X	X	X	X	X
Study drug dispensation			X				X					
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Prior/concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
Mammogram[w]	X									X		X[x]

			Treatment Period (24 W)								EOS/ FU	Early Termination
Study Day	**Screening (-56 Days) (±5)**	**Day -28 (±3)**	**Day 1 (-3)[a]**	**Day 14 (±3)**	**Day 29 (±3)**	**Day 57 (±3)**	**Day 85 (±3)**	**Day 113 (±3)**	**Day 141 (±3)**	**Day 169 (±3)**	**Day 197 (±3)**	
			Baseline[a]							**End of Treatment[b]**	**Follow-Up[c]**	**ET[d]**
PK Sub-Study Assessments[y]												
Telephone call or in-person visit	IP	IP	IP	PC/IH	IP	PC/IH	IP	PC	PC	IP	PC	IP
Serial PTC857 PK blood sampling			X		X		X			X		X
Riluzole PK blood sampling		X	X		X							
Edaravone PK blood sampling		X	X		X							
Sodium phenylbutyrate/taurursodiol PK sampling		X	X		X							
CSF for PK[z]			X		X					X		X
Additional ECGs			X		X							

Abbreviations: ALS, amyotrophic lateral sclerosis; ALS CBS, ALS Cognitive Behavioral Screen; ALSAQ-40, ALS Assessment Questionnaire; ALSFRS-R, ALS Functional Rating Scale-Revised; BMI, body mass index; COVID-19, coronavirus disease 2019; CSF, cerebrospinal fluid; C-SSRS, Columbia-Suicide Severity Rating Scale; ECG, electrocardiogram; EOS, end of study; EQ-5D-5L, five-level European quality of life five-dimensional questionnaire; ET, early termination; FSH, follicle-stimulating hormone; FU, follow-up; ICF, Informed Consent Form; IH, in-home; IP, in-person; LTE, Long-Term Extension; p75NTR, p75 neurotrophin receptor; PC, telephone call; PCR, polymerase chain reaction; PFT, pulmonary function test; PI, Principal Investigator; PK, pharmacokinetic; SAE, serious adverse event

[a] The Baseline Visit will be defined as Day 1 of the study. Baseline Visit procedures may be performed within the 3 days before the day that study drug is first administered. The day in which the first dose of study drug is administered will be Day 1. For subjects taking part in the PK Sub-Study, the PK laboratory tests must be completed on the same day as the first dose of study drug.
[b] Day 169 will be considered the End of Treatment Visit only for those who choose not to enter the LTE Period (Part B).
[c] Follow-up Visit on Day 197 only for subjects who choose not to enter the LTE Period (Part B).
[d] For subjects who terminate early from the study and consent to continued follow-up, the investigator should attempt to gain vital status on the participant and conduct the ALSFRS-R assessment approximately every 28 days for the remaining duration of the study, in accordance with local requirements/regulations.
[e] The Day 29 Visit will be conducted in-person for subjects participating in the PK Sub-Study only.
[f] For the telephone call/in-home visits, where applicable, the ALSFRS-R, C-SSRS, adverse events, and prior/concomitant medications assessments will be conducted via a telephone call. Vital signs assessments and samples for clinical laboratory tests will be collected via a home health nurse during applicable in-home visits at the subject's home during these visits. Subjects may go to the site for an in-person visit at the discretion of the PI. Visits indicated as in-person only may be conducted remotely (ie, in-home) following discussion between the site and the medical monitor. In-person visits that are conducted remotely must include the following assessments, at minimum (if such assessments are typically included during the visit): serum/urine pregnancy test (females only), vital signs, clinical laboratory tests, ALSFRS-R, C-SSRS, adverse events, prior/concomitant medications, sparse PK blood sampling, laboratory biomarkers, and ALSAQ-40.
[g] The ICF for Part B should be provided to the subject at the Day 85 visit to allow the subject adequate time to consider transitioning into Part B.
[h] The ICF for Part B should be signed by the subject at the Day 169 visit if they choose to enroll into Part B.
[i] Women of childbearing potential will have a serum pregnancy test at the initial Screening Visit and then undergo monthly urine pregnancy testing starting from Day 1 prior to randomization through the Follow-up Visit. If a urine pregnancy test is positive, a serum pregnancy test will be conducted for confirmation and dosing should be immediately suspended and evaluated for continued study participation based upon the result of the serum pregnancy test and in discussion with the medical monitor. For months where an on-site visit is not scheduled, subjects will complete a urine pregnancy test at home, if local requirements allow, to be reported to the investigator and documented in the eCRF.
[j] Postmenopausal female subjects only.
[k] Single 12-lead ECG measurements will always be performed prior to the dose of medication administered at the clinic. Subjects should be resting for at least 5 minutes in supine position prior to 12-lead ECG procedures.
[l] Vital signs will consist of measurements of supine blood pressure, heart rate, body temperature, and respiratory rate. Vital sign measurements will be collected after the subject has been resting for 5 minutes. Blood pressure and heart rate measurements will be performed with subjects in a supine position, except when they semi reclined because of study procedures and/or AEs (eg, nausea, dizziness) or if deemed necessary by the PI or designee.
[m] A full physical examination will be completed during Screening Period. A targeted physical examination, including neurological examination, will be completed at all other timepoints.
[n] Blood samples for PK evaluation will be collected from all subjects not participating in the PK Sub-Study at predose (prior to the first dose of the day) and at 1 hour (±15 minutes) and 4 hours (±1 hour) post-dose on Day 1 and Day 85. The first 36 subjects that enroll in the study will have a 6-hour (±1 hour) post-dose blood sampling on Day 1 and Day 85; this 6-hour post-dose blood sampling must be at least 2 hours following the 4-hour post-dose blood sampling. Subjects enrolled after the first 36 subjects may optionally have the 6-hour (±1 hour) post-dose blood sampling on Day 1 and Day 85, if feasible. Time of last dose of study drug prior to the study visit should be recorded. On all other visits with blood samples for PTC857 PK, blood samples will only be collected predose. If any SAEs are observed, ad hoc PK sample(s) may be collected, if possible, to measure the levels of PTC857, riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol to determine the causality.
[o] Clinical laboratory tests will consist of fasting lipid profile, hematology, coagulation, blood chemistry, and urinalysis. Urinalysis will only be performed at in-person clinic visits (ie, Screening, Day 1, Day 85, and Day 169).
[p] Clinical laboratory tests on Day 14 will consist only of a fasting lipid profile. Hematology and blood chemistry will not be evaluated during this visit.
[q] It is the intention of the protocol that all subjects will consent to undergo lumbar punctures. Cerebrospinal fluid samples for biomarkers and PK will be collected at predose (first dose of the day) on both the Baseline Visit and the Day 169 Visit; lumbar punctures on these days may be conducted up to 5 days prior to the Baseline Visit and the Day 169 Visit. CSF biomarkers will be assessed as listed in Section 7.1.7.
[r] The lumbar puncture that is required may be skipped for an individual subject if the PI deems it appropriate, and after discussion with the medical monitor.
[s] Laboratory (blood and urine) biomarkers (aligned with the timing for the clinical laboratory tests) as per Section 7.1.7. Urine creatinine should be tested when urine p75NTR is sampled.
[t] The ALSFRS-R assessment must be performed by a certified rater.
[u] Pulmonary function tests will assess slow vital capacity and Sniff Nasal Inspiratory Pressure. Forced vital capacity, vital capacity, forced expiratory volume, and maximum inspiratory pressure will not be measured. Refer to the laboratory manual for specific requirements at Screening. Oxygen saturation, respiratory rate, and heart rate will be assessed in both upright and supine positions. The Sniff Nasal Inspiratory Pressure test will assess nasal pressure in each unilaterally occluded nostril during a maximal sniff to assess inspiratory muscle strength.
[v] During the Screening Visit (Day -56), the "baseline" version of the C-SSRS will be administered. At all other visits, the "since last visit" version will be administered.
[w] Mammograms to be conducted for female subjects only. A mammogram is not required during the Screening Period if the subject has had a negative breast cancer imaging status (ie, not considered clinically abnormal and/or requiring further evaluation/treatment) within 6 months prior to the Screening Visit. If clinically indicated, an ultrasound or MRI may also be conducted for breast cancer imaging, per the investigator's discretion. If a subject cannot complete the Screening mammogram or any necessary follow-up procedures during the Screening Period, the Screening Period may be extended after discussion and agreement with the medical monitor.
[x] Women who have been exposed to study drug for at least 3 months and discontinue treatment before reaching the Day 169 Visit will have breast cancer surveillance imaging performed at the Early Termination Visit. Women who have been exposed to study drug for <3 months and discontinue treatment before reaching the Day 169 Visit may have breast cancer surveillance imaging performed at the investigator's discretion.
[y] The following additional procedures will be conducted only in subjects taking part in the optional PK Sub-Study:

- Blood samples for riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol PK will be collected at Day -28 predose (prior to the first dose of riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol on Day -28) and 1 hour (±15 minutes) post-dose. Subjects should be instructed not to take riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol on Day -28 prior to the study visit.
- Blood samples for PTC857, riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol PK will be collected from those subjects taking part in the PK Sub-Study at predose (prior to the first dose of the day) and at 1, 2, 4, 5, 6, 8, and 12 hours (±15 minutes) (prior to the second dose) post-dose on Day 1 and Day 29. Blood samples for PTC857 PK only will be collected predose (prior to the first dose of the day) and at 1 hour (±15 minutes) and 4 hours (±1 hour) post-dose on Day 85, and predose only at all subsequent visits with blood PK sampling. Subjects should be instructed not to take riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol on Day 1 or Day 29 prior to the study visit. Time of last dose of study drug and riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol prior to the study visit should be recorded.
- Additional samples of predose (trough) CSF will be collected for PK evaluation at all lumbar puncture timepoints.
- Additional ECG measurements will be performed at the time of the 4-hour and 8-hour post-dose PK samples (±15 minutes from PK sample blood draw).

[z] The lumbar punctures for CSF collection in the PK Sub-Study may occur within 5 days prior to or after the scheduled visit day.

Schedule of Assessments for Long-Term Extension Period (Part B)

	Long-Term Extension Period						EOS/ Follow-Up	Early Termination
Study Day	**Day 169 (±3)**	**Day 183 (±3)**	**Day 197 (±3)**	**Day 225 (±3)**	**Day 253 (±3)**	**Day 365 (±5)**	**Day 393 (±3)[a]**	**ET[b]**
						End of Treatment[c]		
Telephone call/in-home or in-person visit[d]	IP	PC/IH[d]	PC/IH[d]	PC/IH[d]	IP	IP	PC	IP
Informed consent	X[e]				X[f]	X[g]		
Weight and BMI					X	X		X
Serum/urine pregnancy test (female subjects only)[h]			◄ (Monthly [Days 197, 225, 253, 281, 309, 337, and 365]) ►				X	X
12-Lead ECG[i]					X	X		X
Vital signs[j]			X	X	X	X		X
Physical exam[k]					X	X		X
PK blood sample[l]					X	X		X
Clinical laboratory tests[m]		X[n]	X	X	X	X		X
Laboratory biomarkers (blood and urine)[o]					X	X		X
ALSFRS-R[p]			X	X	X	X	X	X
PFTs[q]					X	X		X
ALS CBS					X	X		X
Modified Norris Scale					X	X		X
ALSAQ-40					X	X		X
EQ-5D-5L					X	X		X
C-SSRS[r]			X	X	X	X	X	X
Study drug dispensation	X				X			
Adverse events		X	X	X	X	X	X	X
Prior/concomitant medications		X	X	X	X	X	X	X
Mammogram[s]	X					X		X[t]

Abbreviations: ALS, amyotrophic lateral sclerosis; ALS CBS, ALS Cognitive Behavioral Screen; ALSAQ-40, ALS Assessment Questionnaire; ALSFRS-R, ALS Functional Rating Scale-Revised; BMI, body mass index; CAFS, Combined Assessment of Function and Survival; CSF, cerebrospinal fluid; C-SSRS, Columbia-Suicide Severity Rating Scale; ECG, electrocardiogram; EOS, end of study; EQ-5D-5L, five-level European quality of life five-dimensional questionnaire; ET early termination; ICF, Informed Consent Form; IH, in-home; IP, In-person; LTE, Long-Term Extension; PC, telephone call; PFT, pulmonary function test; PI, Principal Investigator; PK, pharmacokinetic

[a] Follow-up Visit on Day 393 only for subjects who choose not to enter the Continued LTE Period (Part C).
[b] For subjects who early terminate from the study and consent to continued follow-up, the investigator should attempt to gain vital status on the participant and conduct the ALSFRS-R assessment approximately every 28 days for the remaining duration of the study, in accordance with local requirements/regulations.
[c] Day 365 will be considered the End of Treatment Visit only for those who choose not to enter the Continued LTE Period (Part C).
[d] For the telephone call/in-home visit, where applicable, the ALSFRS-R, C-SSRS, adverse events, and prior/concomitant medications assessments will be conducted via a telephone call. Vital signs assessments and samples for clinical laboratory tests will be collected via a home health nurse during applicable in-home visits at the subject's home during these visits. Subjects may go to the site for an in-person visit at the discretion of the PI. Visits indicated as in-person only may be conducted remotely (ie, in-home) following discussion between the site and the medical monitor. In-person visits that are conducted remotely must include the following assessments, at minimum (if such assessments are typically included during the visit): serum/urine pregnancy test (females only), vital signs, clinical laboratory tests, ALSFRS-R, C-SSRS, adverse events, prior/concomitant medications, sparse PK blood sampling, laboratory biomarkers, and ALSAQ-40.
[e] Subjects who decide to enter the LTE Period should sign the consent form before the start of the LTE Period.
[f] The ICF for Part C should be provided to the subject at the Day 253 visit to allow the subject adequate time to consider transitioning into Part C.

[g] The ICF for Part C should be signed by the subject at the Day 365 visit if they choose to enroll into Part C.
[h] Women of childbearing potential will undergo monthly urine pregnancy testing on Days 197, 225, 253, 281, 309, 337, and 365, and at the Follow-up Visit. If a urine pregnancy test is positive, a serum pregnancy test will be conducted for confirmation and dosing should be immediately suspended and evaluated for continued study participation based upon the result of the serum pregnancy test and in discussion with the medical monitor. For months where an on-site visit is not scheduled, subjects will complete a urine pregnancy test at home, if local requirements allow, to be reported to the investigator and documented in the eCRF.
[i] Single 12-lead ECG measurements will always be performed prior to the dose of medication administered at the clinic. Subjects should be resting for at least 5 minutes in supine position prior to 12-lead ECG procedures.
[j] Vital signs will consist of measurements of supine blood pressure, heart rate, body temperature, and respiratory rate. Vital sign measurements will be collected after the subject has been resting for 5 minutes. Blood pressure and heart rate measurements will be performed with subjects in a supine position, except when they semi reclined because of study procedures and/or AEs (eg, nausea, dizziness) or if deemed necessary by the PI or designee.
[k] A targeted physical examination, including neurological examination, will be completed at all timepoints.
[l] Blood samples for PK evaluation will be collected from all subjects at predose only.
[m] Clinical laboratory tests will consist of fasting lipid profile, hematology, coagulation, blood chemistry, and urinalysis. Urinalysis will only be performed at in-person clinic visits (ie, Day 253 and Day 365).
[n] Clinical laboratory tests on Day 183 will consist only of a fasting lipid profile. Hematology and blood chemistry will not be evaluated during this visit.
[o] Laboratory (blood and urine) biomarkers (aligned with the timing for the clinical laboratory tests) as per Section 7.1.7. Urine creatinine should be tested when urine p75NTR is sampled.
[p] The ALSFRS-R assessment must be performed by a certified rater.
[q] Pulmonary function tests will assess slow vital capacity and Sniff Nasal Inspiratory Pressure. Forced vital capacity, vital capacity, forced expiratory volume, and maximum inspiratory pressure will not be measured. Oxygen saturation, respiratory rate, and heart rate will be assessed in both upright and supine positions. The Sniff Nasal Inspiratory Pressure test will assess nasal pressure in each unilaterally occluded nostril during a maximal sniff to assess inspiratory muscle strength.
[r] At all visits, the Since Last Visits C-SSRS will be administered.
[s] Mammograms to be conducted for female subjects only. If clinically indicated, an ultrasound or MRI may also be conducted for breast cancer imaging, per the investigator's discretion.
[t] Women who have been exposed to study drug for at least 3 months and discontinue treatment before reaching the Day 365 Visit will have breast cancer surveillance imaging performed at the Early Termination Visit. Women who have been exposed to study drug for <3 months and discontinue treatment before reaching the Day 365 Visit may have breast cancer surveillance imaging performed at the investigator's discretion.

Schedule of Assessments for Continued Long-Term Extension Period (Part C)

	Continued LTE Period										EOS/ Follow-Up
Study Day/ Recurring Visit[a]	**Day 365 (±5)**	**Day 449 (±7)**	**Day 533 (±7)**	**Day 617 (±7)**	**Day 701 (±7)**	**Day 785 (±7)**	**Day 869 (±7)**	**Day 953 (±7)**	**Day 1037 (±7)**	**Day 1121 (±7)**	**Day 1149 (±3)**
										End of Treatment /ET[b]	
Telephone call/in-home or in-person visit[a]	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	PC
Informed consent	X[c]										
Serum/urine pregnancy test (female subjects only)[d]	◄ (Monthly [Days 393, 421, 449, 477, 505, 533, 561, 589, 617, 645, 673, 701, 729, 757, 785, 813, 841, 869, 897, 925, 953, 981, 1009, 1037, 1065, 1093, and 1121]) ►										X
Weight and BMI			X		X		X		X	X	
12-Lead ECG[e]			X		X		X		X	X	
Vital signs[f]			X		X		X		X	X	
Physical exam[g]			X		X		X		X	X	
Clinical laboratory tests[h]			X		X		X		X	X	
ALSFRS-R[i]		X	X	X	X	X	X	X	X	X	X
ALSAQ-40		X	X	X	X	X	X	X	X	X	
C-SSRS[j]		X	X	X	X	X	X	X	X	X	X
Study drug dispensation	X	X	X	X	X	X	X	X	X		
Adverse events		X	X	X	X	X	X	X	X	X	X
Prior/concomitant medications		X	X	X	X	X	X	X	X	X	X
Mammogram[k]	X				X					X[l]	

Abbreviations: ALSAQ-40, ALS Assessment Questionnaire; ALSFRS-R, ALS Functional Rating Scale-Revised; C-SSRS, Columbia-Suicide Severity Rating Scale; ECG, electrocardiogram; EOS, end of study; ET, early termination; IP, in-person; PC, telephone call; PI, Principal Investigator

[a] All study visits during the Continued LTE Period should be conducted on-site. Visits indicated as in-person only may be conducted remotely (ie, in-home) following discussion between the site and the medical monitor. In-person visits that are conducted remotely must include the following assessments, at minimum (if such assessments are typically included during the visit): serum/urine pregnancy test (females only), vital signs, clinical laboratory tests, ALSFRS-R, C-SSRS, adverse events, prior/concomitant medications, and ALSAQ-40.

[b] For subjects who early terminate from the study and consent to continued follow-up, the investigator should attempt to gain vital status on the participant and conduct the ALSFRS-R assessment approximately every 28 days for the remaining duration of the study, in accordance with local requirements/regulations.

[c] Subjects who decide to enter the Continued LTE Period should sign the consent form before the start of the Continued LTE Period.

[d] Women of childbearing potential will undergo monthly urine pregnancy testing for the duration of the Continued LTE Period, and at the EOS/Follow-up visit or Early Termination. If a urine pregnancy test is positive, a serum pregnancy test will be conducted for confirmation and dosing should be immediately suspended and evaluated for continued study participation based upon the result of the serum pregnancy test and in discussion with the medical monitor. Subjects will complete a urine pregnancy test at home, if local requirements allow, to be reported to the investigator and documented in the eCRF.

[e] Single 12-lead ECG measurements will always be performed prior to the dose of medication administered at the clinic. Subjects should be resting for at least 5 minutes in supine position prior to 12-lead ECG procedures.

[f] Vital signs will consist of measurements of supine blood pressure, heart rate, body temperature, and respiratory rate. Vital sign measurements will be collected after the subject has been resting for 5 minutes. Blood pressure and heart rate measurements will be performed with subjects in a supine position, except when they are semi reclined because of study procedures and/or adverse events (eg, nausea, dizziness) or if deemed necessary by the PI or designee.

[g] A targeted physical examination, including neurological examination, will be completed at all indicated timepoints.

[h] Clinical laboratory tests will consist of hematology, coagulation, blood chemistry, and urinalysis.

[i] The ALSFRS-R assessment must be performed by a certified rater.

[j] At all visits, the Since Last Visits C-SSRS will be administered.

[k] Mammograms to be conducted for female subjects only. If clinically indicated, an ultrasound or MRI may also be conducted for breast cancer imaging, per the investigator's discretion. ALS is a progressive disease, that over time causes severe disability. Thus, if the PI believes that continued breast cancer surveillance is no longer feasible for a specific patent, this test may be skipped, after discussion with the medical monitor.

[l] Women who discontinue from the study prior to the End of Treatment Visit may have breast cancer surveillance imaging performed at the Early Termination Visit, at the investigator's discretion.

12. APPENDIX 2: MISSING DATE AND MISSING ALSFRS-R PROCEDURE

Prior/Concomitant Medications

For the purpose of assessing whether a medication is prior or concomitant, if a medication has a completely missing start date it will be considered a prior medication, and if a medication has a completely missing stop date it will be considered a concomitant medication. If a partial start or stop date occurs, the following imputation process will be implemented:

Partial Missing Start or Stop Date	Imputation for Start Date	Imputation for Stop Date
Day missing, month and year present	• Month and/or year different to month and year of first study drug dose: Impute day with "01" • Month and/or year same as month and year of first study drug dose: Impute day with same day as first dose of study drug.	Impute day with last day of the month
Day and month missing, year present	• Year different to year of first study drug dose: Impute day and month with "01JAN" • Year same as year of first study drug dose: Impute month and day with same month and day as first dose of study drug.	Impute day and month with "31DEC"
Month missing, day and year present	• Year different to year of first study drug dose: Impute month with "JAN" • Year same as year of first study drug dose: Impute month with same month as first dose of study drug.	Impute with "DEC"
Caveats	• If any imputed start date leads to a start date that is after the stop date, then the start date will be imputed with the date of the stop of medication. • No stop date will be imputed if the treatment is ongoing.	

Adverse Events

For the purpose of assessing whether an AE is treatment emergent, if an AE has a completely missing start and stop date, it will be considered treatment emergent; if the stop date is not missing, but the start date is completely missing, it will be considered treatment emergent unless the stop date occurs prior to the first dose of study drug.

For assessing treatment emergence or for calculation of duration of AE, if a partial start or stop date occurs, the following imputation process will be implemented:

Partial Missing Start or Stop Date	Imputation for Start Date	Imputation for Stop Date
Day missing, month and year present	• Month and/or year different to month and year of first study drug dose: Impute day with "01". • Month and/or year same as month and year of first study drug dose: Impute day with same day as first dose of study drug.	Impute day with last day of the month
Day and month missing, year present	• Year different to year of first study drug dose: Impute day and month with "01JAN". • Year same as year of first study drug dose: Impute month and day with same month and day as first dose of study drug.	Impute day and month with "31DEC"

Partial Missing Start or Stop Date	Imputation for Start Date	Imputation for Stop Date
Month missing, day and year present	• Year different to year of first study drug dose: Impute month with "JAN". • Year same as year of first study drug dose: Impute month with same month as first dose of study drug.	Impute with "DEC"
Caveats	• If any imputed start date leads to a start date that is after the stop date, then the start date will be imputed with the date of the stop of AE.	

ALSFRS-R incomplete / missing data

This section describes visit level imputations performed prior to the multiple imputation procedure.

If 1 item is missing within a domain (e.g. Bulbar), then the domain item will be inputted using the following formula:

Imputed domain score = mean of non-missing items multiplied by 3.

If item 12 on the ALSFRS score is missing, and the patient is not reported as having received non-invasive ventilation at the time of the visit (concomitant procedures CRFs) then item 12 will be imputed as a 4.

Total Score:

If a whole domain is missing or two items are missing, then the total ALSFRS-R total score will be imputed from the remaining 3 domains as the mean of all answered item scores in the 3 domains x 12. Total score will not be imputed if more than 1 domain has 2 or more missing items.

13. APPENDIX 3: TRICALS RISK PROFILE CALCULATOR

TRICALS Risk Profile Calculator, v2.01 (2022-02-20), Source: https://tricals.shinyapps.io/risk-profile/

The TRICALS Risk Profile is a prognostic summary value of seven patient characteristics and can be used for patient selection in clinical trials. The Risk Profile Calculator estimates the TRICALS Risk Profile.

References

1. Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17:423-433
2. van Eijk RPA, Westeneng HJ, Nikolakopoulos S, et al. Refining eligibility criteria for amyotrophic lateral sclerosis clinical trials. Neurology 2019;92:451-460
3. van Eijk RPA, Nikolakopoulos S, Roes KCB et al. Innovating clinical trials for amyotrophic lateral sclerosis: challenging the established order. Neurology 2021

Estimation of the TRICALS Risk Profile

The TRICALS Risk Profile is based the ENCALS (European Network to Cure ALS) survival prediction model, a cross-validated model to predict the composite endpoint death or respiratory insufficiency based on data of 11,475 patients with ALS. Patient selection is based on the linear predictor of the model and not on the absolute risk predictions as (1) the baseline hazard significantly varies across countries, and (2) the ENCALS model estimates the absolute survival probability from disease onset rather than trial enrolment. The linear predictor of the model, hereafter stated as TRICALS Risk Profile, can be conceptualized as a relative summary of prognostic information. The risk profile indicates how patients compare to each other (i.e. who is faster or slower progressing than average) without estimating the absolute survival time or probability.

The TRICALS Risk Profile is calculated as:

$$\boldsymbol{Risk\ profile} = (0.474 \times VC_t) - (2.376 \times DxDelay_t) - (1.839 \times \Delta FRS_t) - (0.264 \times Age_t) + (0.271 \times Bulbar) + (0.238 \times Definite) + (0.415 \times FTD)$$

Where *t* represents the non-linear transformations for continuous variables, VC the vital capacity (in %predicted), Age is age at onset in years, diagnostic delay (DxDelay) in months, FTD the presence of frontotemporal dementia and ΔFRS (points per month) is the progression rate of the ALS functional rating scale (ALSFRS-R).

$$Age_{transf.} = \left(\frac{Age\ at\ onset}{100}\right)^{-2} \qquad DxDelay_{transf.} = \left(\frac{DxDelay}{10}\right)^{-\frac{1}{2}} + \ln\left(\frac{DxDelay}{10}\right)$$

$$\Delta FRS_{transf.} = (\Delta FRS + 0.1)^{-\frac{1}{2}} \qquad VC_{transf.} = \left(\frac{VC}{100}\right)^{-1} + \left(\frac{VC}{100}\right)^{-\frac{1}{2}}$$

$$\Delta FRS_{transf} = \left(48 - FRS_{screening}\right) \div DxDelay$$

14. APPENDIX 4: SAS CODE

Mixed Model Repeated Measure

The following is example SAS syntax for producing least squares estimates, for the MMRM analysis:

```
proc mixed data=ALSFRS_LONG method=reml;
   class ID TRTRND(ref="0") STADCARE(ref="3") CHANGE(ref="0") TIME(ref="4");
   model DELFRS = TIME VALUEBL CHANGE STADCARE TIME*TRTRND TIME*VALUEBL TRP
   TIME*TRP / ddfm=kr solution;
   repeated TIME / type=UN subject=id;
   lsmeans trtrnd / pdiff=control('0' '5')cl;
   run;
```

where `id` is the subject identifier, `trtrnd` is the randomized treatment arm, `stadcare` is a factor variable for the SoC stratification group at baseline, `change` is the amount of change in the subject's ALSFRS-R score during the Screening Period, `time` is the categorical variable for study visit, `Valuebl` is the baseline ALSFRS-R score, `TRP` represents the TRICALS risk profile at baseline, and the response variable `DELFRS` is the change from baseline in ALSFRS-R score.:

ANCOVA with Multiple Imputation

SAS code for the ALSFRS-R endpoint at 24 weeks:

```
proc mi data = ALSFRS_DATA out = ALSFRS_DATA_OUT
   seed = 20221219 nimpute = 100 noprint;
   class trtrnd stadcare change sex;
   var trtrnd Valuebl change stadcare sex age trp
   ALSFRS_R_4 ALSFRS_R_8 ALSFRS_R_12 ALSFRS_R_16 ALSFRS_R_20 ALSFRS_R_24;
   fcs reg(ALSFRS_R_4 ALSFRS_R_8 ALSFRS_R_12 ALSFRS_R_16 ALSFRS_R_20
   ALSFRS_R_24);
   run;
```

The next set of code performs the analyses for the ALSFRS-R 24-week endpoint:

```
proc reg data=ALSFRS_DATA_OUT  utset=outreg covout noprint;
   model ALSFRS_24 = trtrnd Valuebl change stadcare TRP;
   by _Imputation_;
   run;
```

This step will combine the results:

```
proc mianalyze data=outreg;
   modeleffects Intercept trtrnd Valuebl change stadcare TRP;
   run;
```

Survival Analysis

The following is sample code for producing a Kaplan-Meier survival plot:

```
  proc lifetest data=survdata plots=survival(cl cb=hw test atrisk);
      time time*status(0);
      strata trtrnd / test=logrand adjust=sidak;
      run;
```

where `time` is the time of event or censoring, `status` is the variable indicating censored (1) or not censored (0), i.e., death observed, `trtrnd` is the treatment allocation variable and is the `TRP` TRICALS risk score. The follow code runs a Cox regression model:

```
proc phreg data=survdata atrisk plots(cl)=(survival cumhaz);
   class trtrnd stadcare TRP;
   model time*status(0)= trtrnd change stadcare / rl=pl;
   run;
```

where `time` is the time of event or censoring, `status` is the variable indicating censored (1) or not censored (0), i.e., death observed, and `trtrnd` is the treatment allocation variable.

15. APPENDIX 5. MAPPING ALSFRS-R TO KING'S STAGE

The King's clinical staging criteria for ALS is defined as a count of involved central nervous system (CNS) regions (Stage 1, 2 or 3), requirement for gastrostomy or noninvasive ventilation (Stage 4) and death (Stage 5).

Stage	Definition
1	Functional involvement of one CNS region (symptom onset)
2	Functional involvement of two CNS regions
3	Functional involvement of three CNS regions
4	Need for gastrostomy (4A) or non-invasive ventilation (4B)
5	Death

Per the study's eligibility criteria, there will be no subjects with Stages 4 or 5

1. Bulbar region is involved: If the subject dropped any points on any of the three ALSFRS-R questions regarding speech (question 1), salivation (question 2) or swallowing (question 3).
2. Upper limb region is involved: If the subject dropped any points on either of the two ALSFRS-R questions regarding hand function, which are handwriting (question 4) and ability to cut food and handle utensils (question 5A).
3. Lower limb region is involved: If the subject dropped any points on the ALSFRS-R question regarding ability to walk (question 8).
4. Stage 4A attained: If question 5B rather than question 5A, which is only answered by patients without a gastrostomy.
5. Stage 4B reached: If a patient scored 0 points on question 10 or less than 4 points on question 12.

16. REVISION HISTORY

Version Number	Date	Description
1.0	25 SEPTEMBER 2024	Initial release