CLINICAL PROTOCOL

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)

PTC857-CNS-001-ALS

14 JUNE 2024

VERSION 6.0

PTC THERAPEUTICS, INC.
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PROTOCOL IDENTIFIERS AND STUDY PERSONNEL

Project Code	PTC857
Therapeutic Area	Amyotrophic Lateral Sclerosis (ALS)
PTC Therapeutics Substance Identifier	PTC857
Protocol Number	PTC857-CNS-001-ALS
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Protocol Title	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)
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The eSignature Page is located on the last page.

PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

I have read the protocol document and, on behalf of my institution, agree to comply with the protocol and all applicable regulations.

Principal Investigator **Date**

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SYNOPSIS

Name of Sponsor/Company: PTC Therapeutics, Inc. (PTC)		
Name of Investigational Product: PTC857		
Name of Active Ingredient: PTC857		
Protocol Number: PTC857-CNS-001-ALS	**Phase:** 2	**Regions:** 1. Global

Title of Study:

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)

Study Period:

The entire study duration for each subject is approximately 172 weeks:

- Screening Period: Approximately 8 weeks
- Treatment Period (Part A): Approximately 24 weeks
- Long-Term Extension (LTE) Period (Part B): Approximately 28 weeks
- Continued LTE Period (Part C): Approximately 108 weeks
- Post-Treatment Follow-Up Period: Approximately 4 weeks

Objectives:

Primary Objective

To evaluate the efficacy of PTC857 in reducing disease progression in subjects with amyotrophic lateral sclerosis (ALS).

Secondary Objectives

The secondary objectives of the 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

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 mechanistically- 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

Long-Term Extension Period Objectives

The objectives of the LTE Period of the 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

Continued Long-Term Extension Period Objectives

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

Endpoints:

Primary Endpoint

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

Secondary Endpoints

The secondary endpoints of the study are the following:

- Subject ranks based on the combined assessment of ALSFRS-R and survival 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 Treatment Period
- 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

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

Long-Term Extension Period Endpoints

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

Continued Long-Term Extension Period Endpoints

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

Study Design and Methodology:

This is a Phase 2, randomized, double-blind, placebo-controlled, parallel study to assess the effects of PTC857 treatment in adult male and female subjects diagnosed with ALS.

The objectives of the study are to evaluate the efficacy, safety, tolerability, PK, and effects on biomarkers of PTC857 therapy in subjects with ALS. The study consists of 5 periods: Screening, Treatment, LTE, Continued LTE, and Follow-Up.

Screening Period

After the written informed consent is signed, all subjects will enter the Screening Period. The Screening Period will last approximately 8 weeks. Subjects will complete all activities as per the Schedule of Assessments. All subjects who complete the full Screening Period and continue to meet the inclusion and exclusion criteria of the study will be eligible to enter the Treatment Period.

Treatment Period (Part A)

At the end of the Screening Period, subjects will return to the study center for the Baseline Visit. All subjects will be assessed to confirm that they still meet the inclusion and exclusion criteria of the study. All subjects who return to the study center and maintain study eligibility at completion of the Screening Period will be randomized to 1 of 2 treatment groups: PTC857 (250 mg BID) or matching placebo at a 2:1 ratio with 2 stratification factors:

(1) Amount of change in ALSFRS-R score during the Screening Period by points total loss:
    a. <1
    b. 1-2
    c. 3-4
    d. >4
(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

Subjects will undergo ALSFRS-R assessment during the Screening Period to determine whether they will be included in the ITT1 Analysis Set.

Study visits will occur as described in the Schedule of Assessments and will include efficacy, PK, biomarker effects, and safety assessments. Efficacy assessments will include evaluation of disease progression, motor/limb and bulbar function, neuropsychological function, and respiratory function. Pharmacokinetic assessments will consist of blood and CSF sampling, and biomarker assessments will consist of blood, urine, and CSF sampling. Safety assessments consist of collecting information on TEAEs, TESAEs, clinical laboratory tests (hematology, serum chemistry, urinalysis), C-SSRS, physical examination, vital signs, and 12-lead ECGs.

At the successful completion of the Treatment Period, all subjects will be offered the opportunity to enter the LTE Period of the study. Those who choose to enter will continue treatment with PTC857 (250 mg BID) as described below. 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.

Long-Term Extension Period (Part B)

Subjects who enter the LTE Period of the study will continue treatment for an additional 28 weeks. All subjects will be treated with open-label PTC857 (250 mg BID) during the LTE Period. Subjects will remain blinded to their original treatment from the Treatment Period (Part A).

Subjects will complete all activities as per the Schedule of Assessments. 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.

Continued Long-Term Extension Period (Part C)

Subjects who enter the Continued LTE Period (Part C) will continue treatment for an 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).

Subjects participating in the Continued LTE Period will complete all activities as per the Schedule of Assessments. 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.

Sample Size Justification:

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 2.5 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. Data from the 2 recently concluded clinical studies in ALS reported overall death rates of 5% and 0% (Edaravone (MCI-186) ALS 19 Study Writing Group 2017, Paganoni 2020). In addition, the edaravone study reported a standard deviation of 5.3 points for the ALSFRS-R score at Week 24, among treated subjects.

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 sampling for missing data is not planned.

Number of Subjects (Planned):

The study is targeted to have approximately 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).

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.

Diagnosis and Main Criteria for Inclusion:

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:
   a. Onset of the first symptom leading to the diagnosis of ALS ≤24 months at the time of the initial Screening Visit
   b. 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 (refer to the laboratory manual for specific requirements)
5. Subjects or their designee (ie, 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 (ie, 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 the study drug
10. Willing and able to comply with all protocol procedures
11. All chronic concomitant medications (both prescription and over-the-counter [OTC]) and non-pharmacologic therapy regimens, excluding standard-of-care therapy riluzole, edaravone, or sodium phenylbutyrate/taurursodiol (refer to inclusion criterion 13), should be stable and unchanged from 14 days prior to the start of the Screening Period and intend to remain stable and unchanged throughout the course of the study
12. 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
13. Standard-of-care therapy for the treatment of ALS (riluzole, edaravone, or sodium phenylbutyrate/taurursodiol) should be stable and unchanged from 30 (-3) days prior to the start of the Screening Period and intend to remain stable and unchanged throughout the course of the study. Note: should a subject be discontinued from standard-of-care therapy due to removal of the therapy from the market, this will not be considered a protocol deviation.

Main Criteria for Exclusion:

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 cardiovascular/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: The lumbar puncture 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) (ie, 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 (-3) 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. Subject is taking a non-approved form of sodium phenylbutyrate and/or taurursodiol for the treatment of ALS

Investigational Product, Dosage, and Mode of Administration:

Subjects will receive 1 of 2 treatment regimens during the 24-week Treatment Period:

- PTC857 (250 mg) administered orally BID for 24 weeks
- Matching placebo (250 mg) administered orally BID for 24 weeks

All subjects will receive open-label PTC857 250 mg BID during the 28-week LTE Period and during the 108-week Continued LTE Period.

Of note, since this is a double-blind study, all subjects will be given an equal number of bottles of either active drug or placebo to maintain the blind setting.

Duration of Treatment:

All subjects will receive 24 weeks of treatment during the Treatment Period, an additional 28 weeks of treatment in the LTE Period, and an additional 108 weeks of treatment in the Continued LTE Period.

Reference Therapy, Dosage, and Mode of Administration:

Placebo for PTC857 will be administered orally twice a day.

Criteria for Evaluation:

The following efficacy assessments will be collected according to the Schedule of Assessments:

- Disease progression as assessed by the 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 breathing) 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 better function.

- Respiratory function as assessed by PFTs:
  Pulmonary function tests will assess slow vital capacity and Sniff Nasal Inspiratory Pressure (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.

- Motor/limb and bulbar function as assessed by the Modified Norris Scale:
  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.

- Neuropsychological function as assessed by the ALS CBS:
  The ALS CBS is a measure of cognition and behavior in patients with ALS. The cognitive section assesses attention, concentration, tracking/monitoring, and initiation and retrieval. The behavioral section compares changes in personality and behavior since the onset of ALS, as well as mood, pseudobulbar affect, and fatigue.

- Disease-specific measure of health-related quality of life as assessed by ALSAQ-40:
  The ALSAQ-40 is a disease-specific measure of health-related quality of life for ALS. It is specifically used to measure the subjective well-being of patients with ALS and provides scores for 5 scales: physical mobility, activities of daily living and independence, eating and drinking, communication, and emotional reactions.

- Health-related quality of life as assessed by EQ-5D-5L:
  The EQ-5D-5L is used for respondents to rate their own health in 5 different dimensions, including mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

- Blood-, urine-, and CSF-based biomarkers:
  Blood, urine, and CSF biomarker levels will evaluate PTC857 biochemical activity and ability to modulate key aspects of disease pathology.

- Plasma and CSF for PTC857 PK:
  - Blood samples will be collected following the first dose and at steady state to evaluate PTC857 PK in subjects.
  - CSF samples will be collected during the study to assess the change of PTC857 PK in CSF following multiple doses.

Safety:

Safety will be assessed by the occurrence of TEAEs, TESAEs, clinical laboratory tests (hematology, serum chemistry, urinalysis), C-SSRS, physical examination, vital signs, and 12-lead ECG in subjects with ALS on PTC857 versus placebo.

Statistical Methods:

Intent-to-Treat 1 Analysis Set:

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. The ITT1 Analysis Set will be used for analysis of the primary endpoint.

Intent-to-Treat 2 Analysis Set:

All subjects who are randomized after the Screening Period and take at least 1 dose of study drug in the Treatment Period will be included in the ITT2 Analysis Set. Subjects will be analyzed according to their randomized treatment.

Per Protocol Analysis 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. The criteria for inclusion in the PP Analysis Set will be finalized prior to study unblinding and detailed in the Statistical Analysis Plan (SAP).

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.

PK Analysis Set:

All subjects in the ITT2 Analysis Set who have at least 1 measurable plasma or CSF PTC857 concentration will be included in the PK Analysis Set.

The null hypothesis of this study is that the subject ranks based on the composite survival and change from baseline in ALSFRS-R after 24 weeks of treatment are the same in the PTC857 (250 mg BID) and in the placebo versus the alternative that they are different; a 2-sided test at the 5% alpha level will be applied.
The primary efficacy endpoint will be analyzed on the ITT1 Analysis Set at the significance level of 0.05 (2-sided). If $p<0.05$, then the study will be declared positive.

For the primary efficacy endpoint, subject ranks based on the Combined Assessment of Function and Survival (CAFS) ranking method, will be analyzed when all subjects in the ITT1 Analysis Set finish the Treatment Period (Part A). This analysis will be performed using an analysis of covariance (ANCOVA) model following multiple imputation. Subjects who discontinue from the study prior to Week 24 will have their Week 24 ALSFRS-R score imputed using multiple imputation. The ANCOVA model will include the following covariates: treatment arm (PTC857 and placebo), baseline ALSFRS-R score, 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 standard-of-care exposure with rank scores as the response variable. A p value for the difference will be presented.

Safety endpoints (severity and number of TEAEs and TESAEs, change from baseline in clinical laboratory tests, physical examination, vital signs, C-SSRS, and 12-lead ECGs) will be descriptively compared between PTC857 and placebo.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialized Term	Explanation
β-HCG	Beta human chorionic gonadotropin
λ_z	Apparent terminal rate constant
12-HETE	12-Hydroxyeicosatetraenoic acid
15-HETE	15-Hydroxyeicosatetraenoic acid
15-LO	15-Lipoxygenase
4HNE	4-Hydroxynonenal
AE	Adverse event
AESI	Adverse event of special interest
ALS	Amyotrophic lateral sclerosis
ALS CBS	Amyotrophic lateral sclerosis Cognitive Behavioral Screen
ALSAQ-40	40-item Amyotrophic Lateral Sclerosis Assessment Questionnaire
ALSFRS-R	ALS Functional Rating Scale-Revised
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
$AUC_{0\text{-}inf}$	AUC from time zero to infinity
$AUC_{0\text{-}t}$	AUC from time zero to time of last non-zero (measurable or sampled) concentration
BMI	Body mass index
CAFS	Combined Assessment of Function and Survival
C_{avg}	Average concentration over a dosing interval
CL/F	Total body clearance
C_{max}	Maximum observed concentration
CNG	Cynomolgus
COVID-19	Coronavirus disease 2019
CRO	Contract research organization
CSF	Cerebrospinal fluid
C-SSRS	Columbia-Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
CTIS	Clinical Trials Information System
C_{trough}	Plasma concentration on Day 29 prior to the morning dose
CV	Cardiovascular
CYP	Cytochrome P450
DDI	Drug-drug interaction
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EDC	Electronic data capture
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EOS	End of study
EQ-5D-5L	Five-level European quality of life five-dimensional questionnaire
FSH	Follicle-stimulating hormone
FU	Follow-up
GCP	Good Clinical Practice
GGT	Gamma-glutamyltransferase
GPX4	Glutathione peroxidase 4
HDL	High-density lipoprotein

Abbreviation or Specialized Term	Explanation
HETE	Hydroxyeicosatetraenoic acid
IB	Investigator's Brochure
ICF	Informed Consent Form
IEC	Independent ethics committee
IH	In-home
INR	International normalized ratio
IP	In-person
IRB	Institutional Review Board
ITT1	Intent-to-Treat 1
ITT2	Intent-to-Treat 2
IUPAC	International Union of Pure and Applied Chemistry
K_2-EDTA	Dipotassium ethylenediaminetetraacetic acid
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LFT	Liver function test
LTE	Long-Term Extension
MedDRA	Medical Dictionary for Regulatory Activities
NfL	Neurofilament light chain
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OTC	Over-the-counter
p75NTR	p75 neurotrophin receptor
PC	Telephone call
PCR	Polymerase chain reaction
PD	Pharmacodynamic(s)
PFT	Pulmonary function test
PI	Principal investigator
PK	Pharmacokinetic(s)
PP	Per Protocol
PT	Prothrombin time
RBC	Red blood cell
ROS	Reactive oxygen species
RSI	Reference Safety Information
SAE	Serious adverse event
RSL3	RAS-selective lethal
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SNIP	Sniff Nasal Inspiratory Pressure
SOC	System Organ Class
SpO_2	Oxygen saturation
SUSAR	Suspected unexpected serious adverse reaction
$T_{½}$	Apparent terminal half-life
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
T_{max}	Time to reach C_{max}
ULN	Upper limit of normal
UN	Unstructured covariance matrix
USM	Urgent safety measure
WBC	White blood cell
WHO	World Health Organization

Abbreviation or Specialized Term	Explanation
WOCBP	Woman of childbearing potential

1. INTRODUCTION

1.1. Background Information

PTC857 (also known as utreloxastat) is an orally bioavailable small molecule (a proprietary cyclohexadiene-dione compound) being developed by PTC Therapeutics (PTC) for the treatment of neurological diseases characterized by high levels of oxidative stress and mitochondrial pathology, including amyotrophic lateral sclerosis (ALS). In diseases characterized by high levels of oxidative stress, reactive oxygen species (ROS) production outstrips the available supply of glutathione, resulting in depletion of glutathione and ROS-mediated cell injury and cell death. PTC857 functions as an inhibitor of the oxidoreductase 15-lipoxygenase (15-LO) enzyme to reduce oxidative stress and spare reduced glutathione.

This study is intended to assess the efficacy, safety, tolerability, pharmacokinetic (PK), and biomarker effects of PTC857 in adult male and female subjects diagnosed with ALS.

1.2. Name and Description of Investigational Product

The International Union of Pure and Applied Chemistry (IUPAC) chemical name for PTC857 is 2,3,5-trimethyl-6-nonyl-2,5-cyclohexadiene-1,4-dione. The drug substance is a pale yellow to yellow crystalline solid. It is physically and chemically stable in solid state at long-term and accelerated conditions. The drug product for this study is a solubilized formulation of PTC857 at a concentration of 60 mg/mL (unflavored formulation) or 62.5 mg/mL (flavored formulation) filled in amber glass bottles with child-resistant closures. Refer to Section 5.1.1 for additional details.

1.3. Mechanism of Action

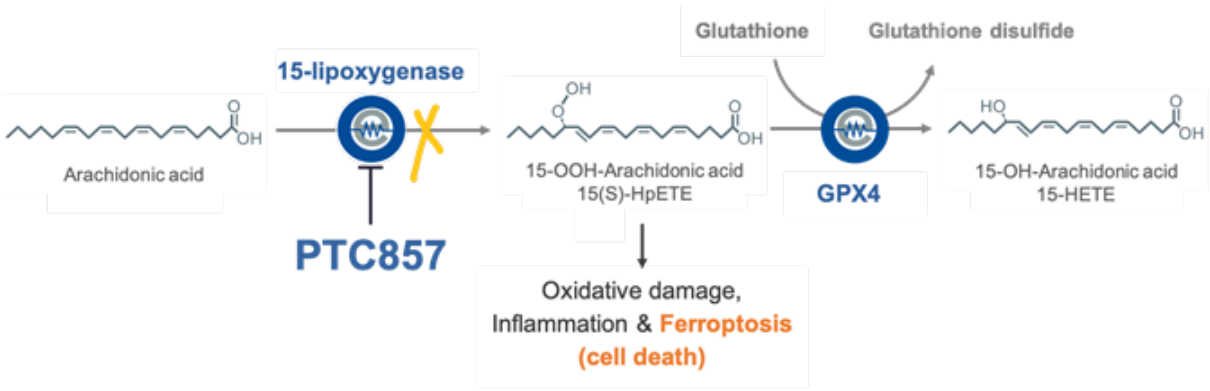
Pharmacologically, PTC857 can be classified as a redox-active inhibitor of ferroptosis. PTC857 functions as an inhibitor of the oxidoreductase 15-LO enzyme to reduce oxidative stress and spare reduced glutathione.

Reduced glutathione is an essential intermediary metabolite that serves as the primary native cellular antioxidant and the cell's primary defense against ROS. It is an important cofactor for the enzyme glutathione peroxidase 4 (GPX4), which regulates ferroptosis by catalyzing the reduction of lipid hydroperoxides to lipid alcohols. The role of GPX4 is important in handling the accumulation of lipid peroxides, thereby preventing oxidative damage and ferroptotic cell death (Masaldan 2019). Under disease conditions, 15-LO is upregulated and produces lipid peroxide products that increase oxidative stress, activate pro-inflammatory glial cells, deplete glutathione, accumulate iron, and ultimately cause ferroptosis (Wenzel 2017, Ye and Stockwell 2017). These biochemical events, in particular the production of oxidized lipids, inflammation, oxidative stress, and glutathione depletion, are known contributors to neuronal cell death and ALS pathogenesis (Masaldan 2019).

Neurodegenerative diseases (Lin and Beal 2006), including ALS, are characterized by high levels of oxidative stress (Ferrante 1997, Barber and Shaw 2010, Maarouf 2012, Reale 2012) and low levels of peripheral and neuronal glutathione (Riederer 1989, Weiduschat 2014).

The schematic representation of the PTC857 mechanism of action is presented in Figure 1.

Figure 1: Proposed Mechanism of Action of PTC857



Abbreviations: 15-HETE, 15-hydroxyeicosatetraenoic acid; GPX4, glutathione peroxidase 4

1.4. Findings From Nonclinical and Clinical Studies

1.4.1. Nonclinical Studies

Nonclinical pharmacodynamic (PD) studies on PTC857 completed to date include 1 enzyme inhibition study, 4 cell pharmacology studies, 1 in vivo PK/PD study, and 2 in vivo efficacy studies. The findings of nonclinical pharmacology studies support the clinical development of PTC857 in neurodegenerative diseases such as ALS.

Results from an in vitro study evaluating the protective activity and potency of PTC857 against ferroptotic cell death in human spinal astrocytes demonstrated that PTC857 prevented RAS-selective lethal compound 3-induced ferroptotic cell death and was 70-fold more potent than edaravone, and riluzole conferred no protection at preventing ferroptotic cell death.

Nonclinical pharmacology studies have demonstrated protection of the neuromuscular junction in an animal model of ALS. In an in vivo study evaluating the efficacy of PTC857 in the SOD1*G93A mouse model of ALS, PTC857 was demonstrated to protect innervation of the neuromuscular junction. In a second study, PTC857 improved grip strength and delayed morbidity of SOD1*G93A mice.

Safety pharmacology studies were conducted in Sprague Dawley rats to assess the effects of PTC857 on respiratory and neurobehavioral function. The effect of PTC857 on cardiovascular (CV) function was assessed in a single-dose study conducted in cynomolgus (CNG) monkeys and in 1-, 3-, and 9-month repeat-dose monkey GLP toxicity studies. These safety pharmacology studies did not identify any risk factors for the administration of PTC857 in subjects.

PTC857 was neither mutagenic nor clastogenic in 2 in vitro (bacterial reverse mutation test and TK6 cell in vitro micronucleus test) and 1 in vivo (rat micronucleus test) genotoxicity studies.

One- and 3-month repeat-dose GLP toxicology studies were conducted in rats and monkeys with PTC857. In the 1-month rat toxicology study, rats were orally administered PTC857 at doses of 0, 100, 300, and 1000 mg/kg/day. The dose-limiting PTC857-associated toxicities observed in the 1-month rat study included moderate increases in prothrombin time in males at 1000 mg/kg/day and microscopic changes in the stomach in both sexes at ≥300 mg/kg/day. Changes in the stomach included mild to moderate submucosal edema, which was observed in non-glandular and glandular sections of the stomach in conjunction with adverse findings in the

mucosa of the non-glandular stomach. The no-observed-adverse effect level was determined to be 100 mg/kg/day. There were no dose-limiting toxicities observed in the 1-month monkey toxicology study or in the 3-month rat and monkey toxicology studies.

A 6-month repeat-dose GLP toxicology study was performed in rats with PTC857 at dose levels of 0, 30, 100, and 200 mg/kg/day. Two of the 14 female rats whose mammary glands were examined in the 200 mg/kg/day group at the end-of-dosing necropsy and 1 of the 4 female rats whose mammary glands were examined in the 100 mg/kg/day group at recovery necropsy had mammary gland adenocarcinomas. The no-observed-effect-level (NOEL) for the mammary gland findings was determined to be 30 mg/kg/day in females. AUC exposure multiples at the NOEL dose in females were approximately 2× anticipated clinical exposures. Mammary gland findings in the female rat were observed at ≥9× AUC exposure multiples compared with anticipated clinical exposures. No findings of adenocarcinomas were observed in male rats at any dose level tested. These findings are likely due to a non-genotoxic mechanism of action, since PTC857 was negative in the Ames mutagenicity and in vitro and in vivo micronucleus genotoxicity assays.

A 9-month repeat-dose GLP toxicology study was performed in monkeys with PTC857 at dose levels of 0, 50, 100, and 300 mg/kg/day. There were no PTC857-related microscopic or macroscopic findings at the end of dosing or recovery necropsies, and no PTC857-related mortality occurred. There were no mammary gland findings in this study.

The phototoxic potential of PTC857 was measured by the relative reduction in viability of BALB/c 3T3 mouse fibroblasts exposed to PTC857 with and without ultraviolet radiation. PTC857 demonstrated phototoxic potential in this assay. The phototoxic potential of PTC857 was further evaluated in an in vivo study in Long-Evans rats administered at dose levels of 0, 30, 100, or 300 mg/kg/day for 3 days. Target skin and eye effects were not observed; there was no evidence of phototoxic potential. Hence, measures to protect against sun and ultraviolet light exposure are not necessary with PTC857 treatment.

An embryo-fetal development study in rats has shown no adverse effects of PTC857 up to the highest dose tested (300 mg/kg/day; ~19× anticipated human exposures at 250 mg BID). An embryo-fetal development study in rabbits showed fetal skeletal abnormalities in the presence of maternal toxicity, which were limited to delayed ossification of the parietals, at the highest dose tested (800 mg/kg/day). The developmental no-observed-adverse-effect level (NOAEL) in rabbits was 300 mg/kg/day (~4.6× anticipated human exposures at 250 mg BID). In a fertility and early embryonic development study in rats, there were no signs of general toxicity observed and there were no effects on reproductive parameters.

1.4.2. Clinical Studies

PTC857 was evaluated in Study PTC857-CNS-001-PD, a 3-part, Phase 1, single-center, double-blind, randomized, placebo-controlled, single- and multiple-ascending-dose, as well as food effect study of PTC857 in healthy volunteers. There were no treatment-emergent serious adverse events (TESAEs), no treatment-emergent adverse events (TEAEs) leading to discontinuation of PTC857, and no TEAEs leading to death.

PTC857 was also evaluated in 6 additional completed Phase 1 studies:

- Study PTC857-CNS-002-HV was a Phase 1, 2-period, crossover, relative bioavailability study of 2 formulations in healthy volunteers.
- Study PTC857-CNS-003-HV was a Phase 1, randomized, open-label, 3-period, 6-sequence crossover study to investigate the effect of food on the PK and safety of PTC857 in healthy volunteers.
- Study PTC857-DDI-101-HV was a Phase 1, open-label, fixed-sequence, 3-part study to investigate the effect of multiple doses of PTC857 on the PK of caffeine (a cytochrome P450 [CYP] 1A2 substrate), midazolam (a CYP3A4 substrate), and bupropion (a CYP2B6 substrate).
- Study PTC857-JPB-102-HV was a Phase 1, open-label, single-dose study to evaluate the PK, safety, and tolerability of PTC857 in healthy Japanese and Caucasian adult subjects.
- Study PTC857-CNS-004-HV was a Phase 1, open-label, multiple-dose, absorption, metabolism, and excretion study in healthy male participants to assess the metabolism, rates and routes of excretion, mass balance, and PK of ^{14}C-PTC857.
- Study PTC857-PAL-104-HV was a Phase 1, single-part, randomized, single-blind study designed to evaluate the taste profile of PTC857 Oral Solution.

There were no TESAEs, no TEAEs leading to discontinuation of PTC857, and no TEAEs leading to death in these studies, with the exception of 1 TEAE of acne that led to discontinuation of a subject in Study PTC857-CNS-003-HV.

Exposure to PTC857 in completed clinical studies included 191 subjects (healthy volunteers) at doses ranging from 100 to 1000 mg. The longest treatment duration was 14 days. The clinical safety data suggest that PTC857 is safe and well tolerated. No TESAEs were reported.

Based on the results of Study PTC857-CNS-003-HV, the PK parameters of PTC857 were higher under low- and high-fat conditions when compared with fasted conditions, but were comparable between the 2 fed states; hence, PTC857 should be taken with food.

Based on the results of Study PTC857-DDI-101-HV, PTC857 can be classified as a weak CYP1A2 inhibitor and a weak CYP3A4 inducer, and CYP2B6 substrates will not be affected by PTC857 co-administration. Hence, dose adjustments are not warranted when drugs metabolized by CYPs 1A2, 3A4, or 2B6 are co-administered with PTC857.

Study PTC857-JPB-102-HV demonstrated a slightly higher, but not clinically meaningful different plasma exposures for Japanese subjects compared with Caucasian participants across all dose levels evaluated; hence, no dose adjustments are required in Japanese subjects.

Study PTC857-CNS-004-HV demonstrated that 88.4% of dosed radioactivity was recovered in combined urine and feces (64.0% in urine and 24.4% in feces) by 264 hours post-dose, with the majority of radioactivity recovered within the first 24 hours post-dose. Metabolism of utreloxastat was extensive and 21 metabolites were detected in plasma, urine, and feces. No unique or disproportionate human metabolites were identified.

Study PTC857-PAL-104-HV demonstrated that the unsweetened/unflavored study drug had poor taste acceptability, and use of sweetener alone or in combination with flavorants improved the taste acceptability.

Refer to the Investigator's Brochure (IB) for additional details on the nonclinical and clinical studies conducted so far.

1.5. Risk/Benefit Assessment

1.5.1. Risk Assessment

Reversible changes in the lipid profile were observed with short-term exposure in healthy human volunteers but not observed in the monkey toxicology studies at exposure multiples 11.8× clinical exposures. Overall, PTC857 has been safe and well tolerated in subjects in the 2 studies of healthy volunteers.

As described in Section 1.4.1, few cases of breast adenocarcinoma were observed in female rats in a 6-month toxicity study. However, there were no mammary gland findings in the 9-month toxicity study in monkeys. The clinical relevance of the findings is yet to be established and will be further investigated in nonclinical studies. Based on the likely non-genotoxic mechanism of action, safety margin to the clinical dose, and low incidence of findings seen, an adequate risk-benefit is present for subjects with ALS in this study.

1.5.2. Potential Benefits of Study Participation

Amyotrophic lateral sclerosis is a neurodegenerative disease that affects the motor neuron system, resulting in progressive paralysis and eventual death due to respiratory failure, typically within 2 to 5 years after onset. Amyotrophic lateral sclerosis is typically diagnosed between the ages of 50 and 60 years. This population is expected to experience continued moderate decline after randomization. Due to the nature of ALS disease progression, it is anticipated that subjects who may not be able to provide ongoing written informed consent throughout the duration, but could provide ongoing nonwritten informed consent, may be included in the study. Hence, nonwritten consent, which may require the presence of a witness, may be provided in accordance with country-specific legislations as applicable. In addition, disease progression may make it difficult to attend on-site visits; hence, subjects may provide informed consent remotely, in accordance with site process and local requirements/regulations. See Section 11.3 for additional information.

Currently, there is no cure for ALS, and treatment of patients with ALS is largely limited to management of symptoms (Petrov 2017). The only approved treatments, in some countries, for ALS are riluzole (Rilutek, administered orally; Tiglutik, oral solution; and Exservan, oral film), edaravone (Radicava, administered intravenously and/or orally), and sodium phenylbutyrate/taurursodiol (Relyvrio, administered orally). As these existing approved therapies are limited in efficacy of altering disease progression, there remains a high unmet medical need for new treatments for ALS.

In in vitro studies, the protective activity and potency of PTC857 against ferroptotic cell death in human astrocytes were demonstrated.

In an ALS mouse model study, PTC857 was demonstrated to protect innervation of the neuromuscular junction. Due to the benefits demonstrated by PTC857 in these pathways for ALS, further studies with PTC857 are warranted in subjects with ALS to understand its potential clinical benefits in this population.

1.5.3. Summary of Safety Risks

There are no important identified or important potential risks for PTC857.

The following potential risks have been recognized to date for PTC857:

- Lipoprotein changes

For further details on each risk, risk mitigation strategies, as well as adverse drug reactions recognized to date, and other important safety topics for PTC857, see the IB.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objective

The primary objective of the study is to evaluate the efficacy of PTC857 in reducing disease progression in subjects with ALS.

2.1.2. Secondary Objectives

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

- Safety and tolerability of PTC857 in subjects with ALS
- 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 of PTC857

2.1.3. 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 mechanistically- 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

2.1.4. Long-Term Extension Period Objectives

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

2.1.5. Continued Long-Term Extension Period Objectives

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

2.2. Endpoints

2.2.1. Primary Endpoints

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

2.2.2. Secondary Endpoints

The secondary endpoints of this study are the following:

- Subject ranks based on the combined assessment of ALSFRS-R and survival 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 TEAEs and 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 Treatment Period
- 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

2.2.3. 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

2.2.4. Long-Term Extension Period Endpoints

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

2.2.5. Continued Long-Term Extension Period Endpoints

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

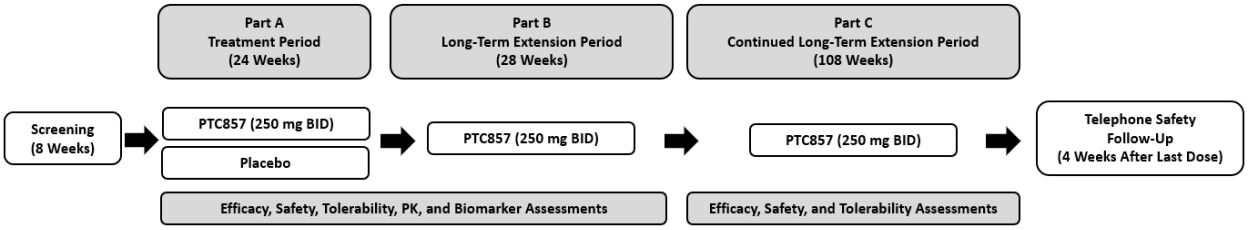
3. STUDY DESIGN

3.1. Overall Study Design

This is a Phase 2, randomized, double-blind, placebo-controlled, parallel study to assess the effects of PTC857 treatment in adult male and female subjects diagnosed with ALS (Figure 2).

The objectives of the study are to evaluate the efficacy, safety, tolerability, PK, and effects on biomarkers of PTC857 therapy in subjects with ALS. The study consists of 5 periods: Screening, Treatment (Part A), LTE (Part B), Continued LTE (Part C), and Follow-Up.

Figure 2: Study Design



Abbreviations: PK, pharmacokinetic

3.2. Treatment Assignment

Screening Period

If a subject is not receiving standard-of-care therapy for the treatment of ALS at the time of entering the Screening Period, the investigator must evaluate whether the individual subject should begin treatment with standard-of-care therapy before entering the clinical study.

After the written informed consent is signed, all subjects will enter the Screening Period. The Screening Period will last approximately 8 weeks. Subjects will complete all activities as per the Schedule of Assessments (Table 1). All subjects who complete the full Screening Period and continue to meet the inclusion and exclusion criteria of the study will be eligible to enter the Treatment Period.

Treatment Period (Part A)

At the end of the Screening Period, subjects will return to the study center for the Baseline Visit. All subjects will be assessed to confirm that they still meet the inclusion and exclusion criteria of the study. Eligible subjects will be randomized 2:1 to 1 of 2 treatment groups: PTC857 (250 mg BID) or matching placebo with 2 stratification factors:

1. Amount of change in ALSFRS-R score during the Screening Period by points total loss:
    a. <1
    b. 1-2
    c. 3-4
    d. >4
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

Subjects will undergo ALSFRS-R assessment during the Screening Period to determine whether they will be included in the ITT1 Analysis Set.

Study visits will occur as described in the Schedule of Assessments and will include efficacy, PK, biomarker effects, and safety assessments. Efficacy assessments will include evaluation of disease progression, motor/limb and bulbar function, neuropsychological function, and respiratory function. Pharmacokinetic assessments will consist of blood and CSF sampling, and biomarker assessments will consist of blood, urine, and CSF sampling. Safety assessments consist of collecting information on TEAEs, TESAEs, clinical laboratory tests (hematology, serum chemistry, urinalysis), C-SSRS, physical examination, vital signs, and 12-lead ECGs.

At the successful completion of the Treatment Period, all subjects will be offered the opportunity to enter the LTE Period of the study. Those who choose to enter will sign an additional Informed Consent Form (ICF) and continue treatment with PTC857 250 mg BID, as described below. 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.

Long-Term Extension Period (Part B)

Subjects who enter the LTE Period of the study will continue treatment for an additional 28 weeks. All subjects will receive open-label PTC857 (250 mg BID) in the LTE Period. Subjects will remain blinded to their original treatment from the Treatment Period (Part A).

Subjects will complete all activities as per the Schedule of Assessments (Table 2). 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.

Continued Long-Term Extension Period (Part C)

Subjects who enter the Continued LTE Period (Part C) will continue treatment for an 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).

Subjects participating in the Continued LTE Period will complete all activities as per the Schedule of Assessments (Table 3). 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.

Pharmacokinetic Sub-Study

Subjects from select sites will participate in the PK Sub-Study. Approximately 36 subjects will take part in an optional PK Sub-Study. These subjects will undergo additional assessments during the study as per the Schedule of Assessments (Table 1).

Table 1: Schedule of Assessments for Screening and Treatment Periods (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

			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]**
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.

Table 2: Schedule of Assessments for the 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.

Table 3: Schedule of Assessments for the 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.

3.3. Number of Subjects

The study is targeted to have approximately 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).

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.

3.4. Sample Size Justification

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 2.5 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. Data from the 2 recently conducted clinical studies in ALS reported overall death rates of 5% and 0% (Edaravone (MCl-186) ALS 19 Study Writing Group 2017, Paganoni 2020). In addition, the edaravone study reported a standard deviation of 5.3 points for the ALSFRS-R score at Week 24, among treated subjects. Assuming approximately 10% of subjects will not meet the ITT1 Analysis Set definition, then approximately 340 subjects will be randomized to ensure that 307 evaluable 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 sampling for missing data is not planned.

If the attrition rate during the study is higher than expected, additional subjects may be enrolled in order to achieve the planned number of evaluable subjects.

3.5. Rationale for Study Duration, Dosing Regimens, and Design

3.5.1. Justification for Study Design and Duration

The 24-week Treatment Period duration was selected in order to assess the effects of exposure of PTC857 on efficacy, safety, and biomarker activity. The study should provide robust data on the risk/benefit profile of PTC857 for the treatment of subjects with ALS.

The Treatment Period duration of 24 weeks was selected based on the following reasons:

- This duration of evaluation is considered appropriate to demonstrate statistically and/or clinically meaningful drug effects and/or potential trends over placebo on disease progression.
- This duration of evaluation is considered adequate to provide a better understanding of the safety and tolerability of PTC857 compared with placebo.
- This duration of evaluation is considered minimally sufficient to provide a better understanding of the relationship between effects on biomarker activity and clinical efficacy of PTC857.

The LTE Period duration of 28 weeks was chosen to provide for a total of 52 weeks of continuous treatment without interruption between the Treatment and LTE Periods with PTC857

to allow for adequate duration to evaluate the long-term safety, tolerability, efficacy, and biomarker effects of PTC857.

The Continued LTE Period duration was chosen to ensure that subjects have the opportunity to continue to receive PTC857 for an additional 108 weeks for continuing assessments of long-term safety and efficacy.

3.5.2. Justification of Selected Doses

The dose of PTC857 (250 mg BID) being evaluated in this study was selected based on nonclinical PK/PD and clinical PK data, as summarized below.

In vitro, PTC857 functions as an inhibitor of the oxidoreductase 15-LO enzyme to reduce oxidative stress and spare reduced glutathione. Such inhibition could be demonstrated via the reduction of 15-LO lipid mediator 15-hydroxyeicosatetraenoic acid (15-HETE) in whole blood. A PK/PD study in mouse indicated that PTC857 reduced the 12/15-LO lipid mediator 12-hydroxyeicosatetraenoic acid (12-HETE) in whole blood following a single-dose of PTC857. The inhibition of 12-HETE production is dose dependent and reached maximum inhibition effects (approximately 60% inhibition of 12-HETE production) at a dose of approximately 100 mg/kg. There was a small increase in inhibition of 12-HETE production in whole blood when the dose was increased from 100 to 300 mg/kg. At a dose of 10 mg/kg PTC857, half of the maximum inhibition (inhibition reduced from 60% to 30%) of 12-HETE production in whole blood was observed.

A preliminary population PK model was developed based on blinded data from the first-in-human study in healthy subjects (Study PTC857-CNS-001-PD) for the single ascending dose cohorts at PTC857 doses of 100, 250, and 500 mg with nominal sampling times. Based on the observed 12-HETE inhibition seen in the mouse model, the projected exposure for 250 mg BID is 1.7× of the exposure for maximum therapeutic effect and 7.7× of the exposure for half maximum therapeutic effect. It is expected that clinical therapeutic effects could be achieved at this dose with sufficient margin of safety (10×) based on the 3-month GLP toxicology study in the most sensitive species (rats). Mammary gland findings in female rats in the 6-month rat toxicology study were observed at ≥9× AUC exposure multiples compared with anticipated clinical exposures. AUC exposure multiples at the NOEL dose in female rats (30 mg/kg/day) for mammary gland findings were approximately 2× anticipated clinical exposures.

In addition, PTC857 250 mg BID for up to 14 days of administration was demonstrated to be safe and well tolerated in a Phase 1 study in healthy subjects. There were no serious adverse events (SAEs), adverse events (AEs) leading to study drug discontinuation, or deaths in the study. In addition, the majority of AEs were mild in intensity and had resolved by the end of the study. The favorable safety profile observed with the use of PTC857 250 mg BID for up to 14 days in healthy subjects further supports the dose selection for this study.

3.5.3. Justification of Selected Study Objectives

The selected clinical efficacy objectives were determined based upon their acceptance and precedence for evaluating and establishing therapeutic value of investigational interventions within the ALS clinical study space. The safety and tolerability objective will be assessed by monitoring of TEAEs, TESAEs, changes in laboratory parameters, and other safety measures during the study. Biomarker efficacy objectives were determined based on the following to

demonstrate and explore effects on target engagement and ALS-associated biomarker activity: (1) existing preclinical evidence of specific targets of engagement by PTC857 and existing clinical evidence of compounds that operate mechanistically similarly to PTC857; and (2) existing preclinical and clinical evidence of biomarker activity implicated and with potential for modulation in ALS and its disease course.

Pharmacokinetic data will be used to characterize the PK of PTC857 in subjects with ALS and explore the relationships, if any, between the PTC857 levels and the safety and efficacy endpoints. Riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol plasma concentration is being measured to help characterize any exposure-response relationship and to inform data interpretation on PK, efficacy, and safety of PTC857. Additionally, efforts will be made to assess the relationships, if any, between exploratory biomarkers and drug concentrations in either plasma, urine, or CSF.

The LTE Period of the study will further evaluate the effects of long-term treatment with PTC857 on safety and tolerability, efficacy, biomarker effects, and PK in subjects with ALS. The Continued LTE Period will further evaluate the effects of long-term treatment with PTC857 on safety and tolerability and efficacy in subjects with ALS.

3.5.4. Justification of Selected Study Population

The inclusion and exclusion criteria for this study were selected to provide a robust population of subjects with ALS with similar features, including, notably, disease progression, who may benefit from treatment with PTC857, while controlling for potential confounders to enable proper evaluation of the product.

Of note, subjects are allowed to continue their current medications, including standard-of-care treatment (riluzole, edaravone, and sodium phenylbutyrate/taurursodiol), to ensure subjects are receiving therapy for their disease if indicated by their treating physician and the medication (riluzole, edaravone, and sodium phenylbutyrate/taurursodiol) regimen is standard and unchanged prior to the study for at least 30 (-3) days and is intended to remain stable and unchanged throughout the course of the study. The concomitant treatment with a combination of edaravone plus sodium phenylbutyrate/taurursodiol is excluded from this study. Furthermore, the use of any non-approved form of sodium phenylbutyrate and/or taurursodiol for the treatment of ALS is also excluded from this study.

3.6. End of Study Definition

A subject is considered to have completed Part A if they have completed the final Part A study visit (ie, either the Day 169 visit for Part A or the follow-up telephone call if they are not participating in the LTE Period [Part B]). The end of Part A is defined as the date of the last scheduled study procedure for the last subject in Part A.

A subject is considered to have completed Part B if they have completed the final Part B study visit (ie, either the Day 365 visit for Part B or the follow-up telephone call if they are not participating in the Continued LTE Period [Part C]). The end of Part B is defined as the date of the last scheduled study procedure for the last subject in Part B.

A subject is considered to have completed Part C if they have completed the final Part C study visit, including the follow-up telephone call after the End of Treatment Visit. The end of Part C is defined as the date of the last scheduled study procedure for the last subject in Part C.

The end of study (EOS) is defined as the date of the last scheduled study procedure of the last subject.

3.7. Criteria for Study Termination/Interruption

The investigator must temporarily interrupt or permanently discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the subject(s). If possible, the medical monitor should be consulted prior to interrupting or discontinuing the study drug. The reason for study drug interruption or premature discontinuation must be documented in the electronic case report form (eCRF), and PTC must be informed.

If a subject terminates treatment with study drug, all efforts should be made to keep the subject in the study and to complete all study activities as per the Schedule of Events, excepting distribution of study drug.

3.8. COVID-19 Risk Mitigation

This study will be conducted in accordance with guidance from applicable regulatory bodies in each country on conducting Phase 2 studies in clinical research centers during the coronavirus disease 2019 (COVID-19) pandemic.

During the entire study, the clinical research center will implement all recommendations issued by the applicable government, including specific guidelines related to clinical research executed in clinical research centers with respect to minimizing the risk of disease spreading (eg, social distancing, disinfection, hygiene, and wearing of personal protection equipment by study staff).

In cases where subjects are not able to attend study visits due to an infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the investigator will discuss with PTC potential mitigation approaches (including, but not limited to, extending the visit window, conducting evaluations via video link or telephone call, allowing for safety procedures to be conducted at a local facility). The rationale (eg, the specific limitation imposed by the SARS-CoV-2 infection that led to the inability to perform the protocol-specified assessment) and outcome of the discussion will be documented in the eCRF.

4. STUDY POPULATION

4.1. Overview

The study population will include adult subjects diagnosed with ALS who satisfy all entry criteria and do not meet any exclusion criteria.

4.2. 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:
    a. Onset of the first symptom leading to the diagnosis of ALS ≤24 months at the time of the initial Screening Visit
    b. Revised El Escorial criteria of either:
        – Clinically definite ALS
        – 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 (refer to the laboratory manual for specific requirements)
5. Subjects or their designee (ie, 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 (ie, 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 the study drug
10. Willing and able to comply with all protocol procedures

11. All chronic concomitant medications (both prescription and over-the-counter [OTC]) and non-pharmacologic therapy regimens, excluding standard-of-care therapy riluzole, edaravone, or sodium phenylbutyrate/taurursodiol (refer to inclusion criterion 13), should be stable and unchanged from 14 days prior to the start of the Screening Period and intend to remain stable and unchanged throughout the course of the study.
12. 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.
13. Standard-of-care therapy for the treatment of ALS (riluzole, edaravone, or sodium phenylbutyrate/taurursodiol) should be stable and unchanged from 30 (-3) days prior to the start of the Screening Period and intend to remain stable and unchanged throughout the course of the study. Note: should a subject be discontinued from standard-of-care therapy due to removal of the therapy from the market, this will not be considered a protocol deviation.

4.3. 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: The lumbar puncture 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) (ie, 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 (-3) 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. Subject is taking a non-approved form of sodium phenylbutyrate and/or taurursodiol for the treatment of ALS

4.4. Treatment Compliance

The study drug will be dispensed based on the Schedule of Assessments (Table 1 and Table 2). The extent of exposure to study medication is defined as the last dose date minus the first dose date +1 day. Compliance will be assessed in terms of the percentage of drug taken relative to the amount that should have been taken during the study. Exposure and compliance will be summarized descriptively.

4.5. Screen Failures

Any subject who does not meet inclusion or exclusion criteria within the defined screening window prior to randomization will be considered a screen failure. Screen failures will be captured in the electronic data capture (EDC) system. Screen failures may be rescreened after consultation with the medical monitor.

4.6. Subject Withdrawal Criteria

Subjects will receive study treatment until protocol-specified study completion or treatment discontinuation for one of the reasons listed below. However, subjects may discontinue study treatment or withdraw their consent to participate in the study at any time without prejudice. In the event that a subject discontinues from study treatment, the subject should be encouraged to continue in the study and complete all study visits and planned study procedures other than administration of study drug.

In the event that a subject completely withdraws from the study prematurely, and unless consent is withdrawn, all efforts must be made to collect efficacy and safety data and to complete the Early Termination Visit. The following conditions require subject discontinuation from all study treatment:

- At their own request or at the request of their legally authorized representative
- If a subject experiences an AE that is deemed related to treatment with PTC857 and in the investigator's or PTC's medical judgment continuation of treatment would be detrimental to the subject
- At the specific request of a regulatory agency or Data and Safety Monitoring Board (DSMB) for termination of treatment of an individual subject or all subjects under the protocol
- Subject participation in another clinical study using an investigational agent or investigational medical device
- Refusal of sexually active fertile subjects (excluding female subjects who have been sterilized) to use medically accepted methods of contraception
- If a subject becomes pregnant
- Significant noncompliance with the protocol in the opinion of the investigator or PTC
- Any situation in which the investigator determines that the subject should not continue in the study

The date PTC857 is discontinued and the reason for discontinuation will be recorded in the source documents and in the eCRF. The medical monitor (and designee) should be informed via email when a subject discontinues study drug.

When PTC857 is discontinued, the investigator is expected to perform all the evaluations according to the subject's original schedule and as required at the Early Termination Visit and any additional evaluations that may be necessary to ensure that the subject is free of untoward effects.

Should a subject be discontinued from standard-of-care therapy due to removal of the therapy from the market, this will not be considered a protocol deviation.

5. STUDY INTERVENTION

5.1. Study Intervention Administration

5.1.1. Study Intervention Description

The treatment(s) to be administered, including the name(s) of all the product(s), the dose(s), the route/mode(s) of administration, and the Treatment Period(s), including the follow-up period(s) for subjects for each investigational product treatment/study treatment group/arm of the study are presented in Table 4. The Schedule of Assessments is presented in Table 1 and Table 2.

Table 4: Investigational Product

	Investigational Product
Product Name	PTC857 oral solution
Dosage Form	Solution
Unit Dosage Strength	4.2 mL of PTC857 Oral Solution, 60 mg/mL (unflavored formulation) OR 4.0 mL of PTC857 Oral Solution, 62.5 mg/mL (flavored formulation) All subjects will receive the unflavored formulation in Part A. Upon completion of Part A, subjects will progressively transition to receive the flavored formulation.
Total Dose	Subjects will receive 1 of 2 treatment regimens during the 24-week Treatment Period: PTC857 250 mg BID or placebo.
Route of Administration	Oral
Physical Description	Clear to translucent yellow to orange liquid
Treatment Period	All subjects will receive 24 weeks of treatment, an additional 28 weeks in the LTE Period, and an additional 108 weeks in the Continued LTE Period.

Abbreviations: LTE, Long-Term Extension

Refer to the IB for additional information.

5.1.2. Dosing and Administration

In all parts of the study, the dosing will take place in the morning and evening, approximately 12 hours apart, with water and a meal (ie, with breakfast and dinner).

Subjects will receive 1 of 2 treatment regimens during the 24-week Treatment Period:

- PTC857 (250 mg) administered orally BID for 24 weeks
- Matching placebo administered orally BID for 24 weeks

Of note, since this is a double-blind study, all subjects will be given an equal number of bottles of either active drug or placebo to maintain the blind setting.

All subjects will receive open-label PTC857 250 mg BID during the 28-week LTE Period and during the 108-week Continued LTE Period.

When clinically indicated, nasogastric and/or percutaneous endoscopic gastrostomy feeding tubes made of polyurethane or silicone may be used for administration of PTC857. Any questions regarding usage of other materials for administration of PTC857 should be discussed with the Medical Monitoring Team.

5.2. Preparation/Handling/Storage/Accountability

5.2.1. Preparation of PTC857 Oral Solution

The drug product will be manufactured under cGMP conditions at a contract facility.

5.2.2. Storage and Accountability

Upon receipt of the study drugs, the responsible pharmacist or his/her designee will inspect all study drugs. Subsequently, he/she must immediately return the enclosed acknowledgment of receipt form, duly completed and signed (the date of receipt must be noted).

The pharmacist or his/her designee is responsible for storage of the study drugs (placebo and PTC857 active pharmaceutical ingredient solution) at the study site in an appropriate secure locked area according to the instructions provided by PTC and/or the manufacturer. Only the pharmacist or his/her designee should handle the study drug.

A Drug Accountability Record must be kept current and should at least contain the following information:

- Subject number for whom the drug was prepared
- Date and initials of the person who prepared the study drug
- Date(s) on which drug was prepared, quantity of the drug prepared, and the relevant lot numbers

The inventory must be available for inspection by the monitor.

After PTC confirmation, all unused investigational material (drugs and packaging) must be returned to PTC or destroyed by the site on termination of the study and after a drug accountability check by the monitor, listing the following:

- All PTC857 administered
- All unused PTC857
- All PTC857 returned at the EOS and the date of return/destroyed by the site

The pharmacist will be responsible for the inventory and accountability of all clinical study material, exercising accepted pharmaceutical practices. An accurate, timely record of the clinical study material will be maintained. The clinical study material and the inventory will be available for inspection by the designated representatives of PTC upon request. The original Drug Preparation Protocol and Drug Accountability Record are considered as source data and will be archived at the site.

5.2.3. Formulation, Appearance, Packaging, and Labeling

5.2.3.1. Formulation and Appearance

The appearance of the drug product is a clear to translucent yellow to orange liquid.

5.2.3.2. Study Drug Packaging

The study drug solution will be packaged in amber glass bottles with child-resistant closures.

5.2.3.3. Study Drug Labeling

The labeling will comply with the applicable (local) laws and regulations.

5.2.4. Handling of Study Drug

Drug supplies must be kept in an appropriate, secure area and stored according to the conditions specified on the drug labels. The site must maintain an accurate record of the shipment and dispensing of study drug(s) on an accountability form.

The pharmacist is responsible for keeping accurate records of the packaged and labeled drug product received from PTC or designee contract facility, and clinical supplies received from PTC or contract facility designee, including, but not limited to, the date received, lot number, amount received, amount returned, and the disposition of all study drug products. Drug accountability records must also be maintained by the site that include the subject's assigned study number, date and amount of study drug dispensed, and relevant lot numbers.

All drug supplies are to be used only for this protocol and not for any other purpose. The responsible person must not destroy any drug labels or unused drug supply. Used and unused drug containers can be destroyed at the site once drug accountability is final and checked by PTC or its deputy and written permission for destruction has been obtained from PTC. A certificate of destruction will be issued and shared with PTC.

5.3. Measures to Minimize Bias: Randomization

This is a double-blind study. The Interactive Web/Voice Response Systems (Interactive Response Technology) will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a subject's intervention assignment is warranted. Subject safety must always be the first consideration in making such a determination. If a subject's intervention assignment is unblinded, the sponsor per the medical monitoring plan must be notified within 24 hours of this occurrence. The outcome of the unblinding (ie, whether the subject was receiving active or placebo) must not be communicated to the sponsor. The date and reason for the unblinding must be recorded.

Sponsor safety staff may unblind the intervention assignment for any subject with an SAE.

5.3.1. Randomization

After the written informed consent is signed, all subjects will enter the Screening Period. The Screening Period will last approximately 8 weeks. Subjects will complete all activities as per the Schedule of Assessments. All subjects who complete the full Screening Period and continue to meet the inclusion and exclusion criteria of the study will be eligible to be randomized and enter the Treatment Period.

Subjects will be randomized 2:1 to either PTC857 (250 mg BID) or 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 Screening Period by points total loss:
    a. <1
    b. 1-2

c. 3-4
d. >4

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

The LTE Period and the Continued LTE Period are both open-label and non-randomized.

The randomization schedules will be incorporated into the interactive response technology system.

5.3.2. Treatment Assignment

All subjects who return to the study center and maintain study eligibility at completion of the Screening Period will be randomized to 1 of 2 treatment groups: PTC857 (250 mg BID) or matching placebo at a 2:1 ratio with 2 stratification factors as outlined in Section 5.3.1. The Schedule of Assessments for the Treatment Period is presented in Table 1.

All subjects who enter the LTE Period of the study will continue treatment for an additional 28 weeks, and all subjects who enter the Continued LTE Period will continue treatment for an additional 108 weeks. All subjects will receive open-label 250 mg BID PTC857 during the LTE Period and during the Continued LTE Period. The Schedule of Assessments for the LTE and Continued LTE Periods are presented in Table 2 and Table 3, respectively.

5.4. Study Intervention and Compliance

Records of study drug used, dosages administered, and intervals between visits are kept during the study. Study drug accountability is performed on an ongoing basis by the study staff and checked by the monitor during site visits and at the completion of the study.

5.5. Concomitant Therapy

If a subject is not receiving standard-of-care therapy for the treatment of ALS at the time of entering the Screening Period, the investigator must evaluate whether the individual subject should begin treatment with standard-of-care therapy before entering the clinical study.

Subjects are permitted to continue their current medications, including standard-of-care treatment (with the exception of the combination of edaravone plus sodium phenylbutyrate/taurursodiol), to ensure they are receiving appropriate therapy for their disease if indicated by their treating physician, so long as the standard-of-care therapy regimen is stable and unchanged prior to the study and is intended to remain stable and unchanged throughout the course of the study. Specific requirements for concomitant therapy are presented herein.

All medications, including OTC treatments, should be kept at a stable and unchanged dose from 14 days prior to the start of the Screening Period and throughout the study. New medications should not be started and/or the dose of a medication should not be changed unless needed to treat an AE or palliative management of progression of symptoms of ALS.

Subjects receiving standard-of-care therapy (riluzole, edaravone, or sodium phenylbutyrate/taurursodiol) for ALS must be on a stable and unchanged dose for at least 30 (-3) days prior to the start of the Screening Period and intend to remain stable and unchanged throughout the course of the study.

The combination of edaravone plus sodium phenylbutyrate/taurursodiol is not permitted for enrollment to this study. Furthermore, the use of any non-approved form of sodium phenylbutyrate and/or taurursodiol for the treatment of ALS is also excluded from this study.

Subjects may not begin standard-of-care therapy (riluzole, edaravone, or sodium phenylbutyrate/taurursodiol) for ALS during the placebo-controlled Treatment Period (Part A).

No other regionally approved treatments for ALS may be ongoing at the start of the Screening Period.

Beginning upon entry to the LTE Period, participants may continue their established standard-of-care treatment and/or initiate treatment with standard-of-care therapy based on their treating physician's discretion where applicable or available without restriction to the number or combination of therapies.

If concomitant medication is needed during the study, this medication must be recorded on the eCRF, stating its generic name, time of administration, dose, route, frequency, duration (start and stop date), and reason for administration.

A list of prohibited concomitant medications during the course of the study is provided in Section 16.1.

6. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.1. Premature Termination or Suspension of the Study

PTC reserves the right to terminate the study at any time. If a study is prematurely terminated or suspended, PTC will promptly inform the investigators, the institutional review boards (IRBs)/independent ethics committees (IECs), and health authorities, as appropriate, and provide the reason(s) for the termination or suspension.

6.2. Participant Discontinuation/Withdrawal From the Study

Subjects may withdraw from the study at any time without prejudice to their future medical care by the physician or at the institution.

The withdrawal of a subject from the study should be discussed where possible with the medical monitor before the subject stops taking the investigational product. If the investigational product is discontinued, the subject should be encouraged to continue in the study and complete all study visits and planned study procedures, other than administration of study drug. If a subject plans to completely withdraw from the study, the Early Termination procedures should be completed.

6.3. Lost to Follow-Up

Subjects are considered lost to follow-up if the attempts to contact the subject are unsuccessful. Efforts must be made on the part of the site to avoid any subject being lost to follow-up during the study. Before subjects are considered lost to follow-up, a minimum of 2 documented telephone contact attempts and 1 certified letter within 6 weeks of the most recent planned study visit must be sent in efforts to contact the subject. After being considered lost to follow-up, a subject's status may be changed if the subject makes contact at a later time provided the study is ongoing.

6.4. Subject's Follow-Up After Study Discontinuation

The subjects will be advised that participation in these investigations is voluntary. Furthermore, the subjects may request that from the timepoint of withdrawal, no more data will be recorded unless otherwise specified by the investigator and consent is provided by the subject (or designee).

Subjects who prematurely discontinue from study drug intake should be encouraged to continue study participation. Subjects should continue to follow all activities as per the Schedule of Events, excepting the dispensation of study drug.

In the case of premature discontinuation after study drug intake, the assessments scheduled for the EOS examination will be performed as soon as possible after study drug intake, as per investigator judgment, unless the subject withdrew informed consent.

Every effort will be made to obtain follow-up information for all subjects whether or not they continue on treatment in the LTE Period or in the Continued LTE Period.

With the subject's consent, PTC will continue to follow-up on the subject's long-term outcomes, as per local regulations.

7. STUDY ASSESSMENT AND PROCEDURES

The scheduled timepoints and study parameters are delineated in the Schedule of Assessments (Table 1 [Part A], Table 2 [Part B], and Table 3 [Part C]).

In case several study procedures are scheduled at the same timepoint, the following sequence should be followed predose: 12-lead ECG, vital signs, PK blood sampling, and blood sampling for clinical laboratory tests. Likewise, for post-dose, the following sequence should be followed: 12-lead ECG, vital signs, PK blood sampling, and blood sampling for clinical laboratory tests.

During the Day 1, Day 85, and Day 169 Visits, the following testing clinical measures should be performed predose: ALSFRS-R, PFTs, ALS CBS, Modified Norris Scale, C-SSRS, and ALSAQ-40. The ALSFRS-R should be the first test performed; all other tests may be performed in any order.

7.1. Efficacy Assessments

Efficacy will be monitored through investigation of the following assessments, which will be collected according to the Schedule of Assessments (Table 1, Table 2, and Table 3).

7.1.1. PFT Assessments

Pulmonary function tests will assess slow vital capacity and Sniff Nasal Inspiratory Pressure (SNIP). 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 SNIP test will assess nasal pressure in each unilaterally occluded nostril during a maximal sniff to assess inspiratory muscle strength.

7.1.2. Modified Norris Scale Assessment

Motor/limb and bulbar function is assessed by the Modified Norris Scale (Oda 1996, Cedarbaum and Stambler 1997, Jenkinson 1999).

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.

7.1.3. ALS Cognitive Behavioral Screen Assessment

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. The behavioral section is composed of questions sensitive to organic brain changes. It consists of a set of questions that compare changes in personality and behavior since the onset of ALS, as well as yes/no questions about mood, pseudobulbar affect, and fatigue.

7.1.4. ALSAQ-40 Assessment

The ALSAQ-40 is a disease-specific measure of health-related quality of life for ALS. It is specifically used to measure the subjective well-being of patients with ALS and provides scores

for 5 scales: physical mobility, activities of daily living and independence, eating and drinking, communication, and emotional reactions.

The ALSAQ-40 is a subjective health measure designed specifically to assess areas of importance in subjects with ALS.

7.1.5. EQ-5D-5L Assessment

The EQ-5D-5L is a measure of quality of life, where respondents rate their own health on 5 different dimensions; each dimension has 5 levels of severity. Dimensions include mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

7.1.6. ALSFRS-R Assessment

Disease progression will be assessed by 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 breathing) 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 better function.

7.1.7. Plasma-, Urine-, and CSF-Based Biomarkers

Plasma- and CSF-based biomarker levels will be performed to evaluate PTC857's biochemical activity and ability to modulate key aspects of disease pathology.

Plasma-based biomarkers will be collected in all subjects. CSF-based biomarkers will only be collected at a subset of clinical sites, as follows:

- Plasma: (target engagement) hydroxyeicosatetraenoic acid panel, (disease-specific biomarkers) NfL and ferritin, and (oxidative stress) 4-hydroxynonenal (4HNE)
- Urine: p75 neurotrophin receptor (p75NTR) fragment extracellular domain normalized to creatinine
- CSF: (disease-specific biomarker) NfL

7.1.8. Plasma and CSF for PTC857 PK

Blood samples will be collected following the first dose and at steady state to evaluate PTC857 PK in subjects.

CSF samples will be collected during the study to assess the change of PTC857 in CSF following multiple doses.

Instruction for blood and CSF sampling, collection, processing, and sample shipment will be provided separately in a laboratory manual.

7.1.8.1. Sparse Blood Sample Collection (Subjects Not in the PK Sub-Study)

All subjects not participating in the PK Sub-Study will undergo sparse blood sampling to contribute toward a population PK model for PTC857. Blood samples 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, an 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.

7.1.8.2. Serial Blood Sample Collection (PK Sub-Study)

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. Blood samples for the determination of PTC857 will be collected in blood collection tubes with dipotassium ethylenediaminetetraacetic acid (K_2-EDTA). Blood sampling for PK will be collected as follows:

- 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.

7.1.8.3. CSF Sample Collection (PK Sub-Study)

CSF samples will be collected predose (first dose of the day) on the Baseline Visit, Day 29 Visit, and the End of Treatment Visit (Table 1). The samples will be tested for biomarkers as per Section 7.1.7 and for PTC857 PK (PK Sub-Study only). Additional samples of predose (trough) CSF will be collected for PTC857 PK evaluation at all lumbar puncture time points.

For subjects not taking part in the PK Sub-Study, the CSF samples will only be tested for biomarkers as per Section 7.1.7 but not for PTC857 PK.

7.1.8.4. Pharmacokinetic Parameters

The following PK parameters will be calculated for PTC857 in plasma when appropriate:

- AUC_{0-t}: Area under the concentration-time curve (AUC) from time zero to time of last non-zero (measurable or sampled) concentration
- AUC_{0-inf}: AUC from time zero to infinity
- C_{max}: Maximum observed concentration
- C_{avg}: Average concentration over a dosing interval
- T_{max}: Time to reach C_{max}. If the maximum value occurs at more than 1 timepoint, T_{max} is defined as the first timepoint with this value
- C_{trough}: Plasma concentration on Day 29 prior to the morning dose
- λ_z: Apparent terminal rate constant calculated by linear regression of the terminal linear portion of the log concentration versus time curve
- $T_{½}$: Apparent terminal half-life
- CL/F: Total body clearance
- Sparse PK sampling collected for subjects not participating in the PK Sub-Study will be used for population PK analysis.

7.1.8.5. Analytical Method

Plasma samples will be analyzed for PTC857, riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol concentration using validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods. Similarly, CSF samples will be analyzed for PTC857 using a validated LC-MS/MS method.

7.1.8.6. Timing for Sampling

The PK blood and CSF sampling for PK timepoints are presented in the Schedule of Assessments for each part of the study (Table 1 and Table 2).

7.1.8.7. Procedures for Sampling

Approximately 4.0 mL of blood for the PK samples will be collected via venipuncture or via an intravenous catheter placed in a vein in the arm following local standard procedures. Approximately 0.5 mL of CSF will be collected via lumbar puncture following standard procedures. Information on equipment and further details on the procedures of the sampling will be documented in a separate PK instruction in the laboratory manual.

7.1.8.8. Labeling

PK blood and plasma tubes will be pre-labeled prior to sample collection. Detailed information regarding collection, processing, handling, and storage will be given in a separate PK instruction in the laboratory manual.

7.1.8.9. Shipping Procedures

The clinical site staff will take care of the shipment of the samples. Details on the procedures on the sample shipment will be documented in a separate PK instruction in the laboratory manual.

7.2. Safety Assessments

Safety will be monitored through assessment of the incidence and severity of TEAEs (AEs that begin after start of study drug), TESAEs, vital signs, weight and height measurements, physical examination findings, C-SSRS, 12-lead ECG, and results of clinical laboratory tests.

Refer to the Schedule of Assessments (Table 1, Table 2, and Table 3) for the timing of each safety assessment.

7.2.1. Vital Signs

Vital sign measurements will be collected after the subject has been resting for 5 minutes.

Single measurements of body temperature, respiratory rate, blood pressure, and heart rate will be measured. Additional vital signs may be taken at any other times if deemed necessary.

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 principal investigator (PI) or designee.

7.2.2. Weight and Height

The subject's body weight will be measured using a calibrated balance. Body weight will be recorded with 1 decimal. The subject's height is measured without wearing shoes. The BMI will be calculated from the weight and height recorded during the Screening Period (based on the following formula):

BMI (kg/m^2)=weight (kg)/height^2 (m^2).

7.2.3. Physical Examination

A complete physical examination will be performed at Screening Period, and a targeted physical examination will be performed in the remainder of the visits. Clinically relevant findings that are observed prior to the Screening Period assessment must be recorded on the relevant Medical History eCRF section. Clinically relevant findings found after study drug initiation and meeting the definition of an AE (new AE or worsening of previously existing condition) must be recorded on the AE section of the eCRF.

7.2.4. 12-Lead Electrocardiogram

The predose 12-lead ECG assessment will be performed prior to administration of the study drug at the clinic visit. In subjects in the PK Sub-Study, an additional 12-lead ECG will be taken at approximately 4 and 8 hours after administration of study drug. Additional ECGs may be taken at any other times if deemed necessary by the PI or designee. Subjects should be resting for at least 5 minutes in supine position prior to ECG procedures.

All ECG tracings will be reviewed by the PI or designee. ECGs will be evaluated and classified as normal/abnormal. In case of "abnormal," the abnormality must be described and its relevance in terms of clinical significance documented.

7.2.5. Clinical Laboratory Assessments

Blood samples will be taken under fasted conditions. Subjects will not be allowed to eat for a period of 8 hours prior to blood sampling or drink (except water) for a period of 4 hours prior to blood sampling.

All clinical laboratory tests listed in Table 5 will be performed as outlined in the Schedule of Assessments (Table 1 and Table 2). In addition, laboratory safety tests may be performed at various unscheduled timepoints if deemed necessary by the PI or designee.

Clinically significant laboratory abnormalities must be reported by the investigator as an AE or SAE as appropriate.

The results of all laboratory tests will be provided to the investigator in order for the investigation to make an assessment of clinical significance, with the exception of the lipid (cholesterol [total], low-density lipoprotein [LDL], high-density lipoprotein [HDL], triglycerides, apolipoprotein A, apolipoprotein B), amylase, and lipase values. The lipid, amylase, and lipase values (both individual subjects and study level) will be monitored regularly for the entirety of the study by the DSMB.

Table 5: Summary of Clinical Laboratory Tests

Hematology	Chemistry	Urinalysis	Others
Hemoglobin	Urea	Urobilinogen	Serum/urine pregnancy test (β-HCG)[b]
Hematocrit	Creatinine	Nitrite	FSH
RBC count	Fasting glucose	pH	Coagulation (PT [sec], PT [INR], aPTT)
Reticulocytes	Sodium	Glucose	
Platelet count	Potassium	Total protein	
WBC count	ALT	Erythrocytes	
Neutrophils	AST	Leukocytes	
Eosinophils	GGT	Ketones	
Monocytes	Bilirubin (total, direct/indirect)	Microscopy[a]	
Basophils	Alkaline phosphatase	Specific gravity	
Lymphocytes	Albumin	Bilirubin	
Mean corpuscular volume	Cholesterol (total)		
	LDL		
	HDL		
	Triglycerides		
	LDH		
	Amylase		
	Lipase		
	Apolipoprotein A		
	Apolipoprotein B		

Abbreviations: β-HCG, beta human chorionic gonadotropin; ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; FSH, follicle-stimulating hormone; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; INR, international normalized ratio; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; PCR, polymerase chain reaction; PT, prothrombin time; RBC, red blood cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WBC, white blood cell

[a] Urine sediment examination will only be performed if there is an abnormality in urinalysis in accordance with clinical laboratory standard procedures.

[b] Required for all female subjects.

Note: Nasal and throat mucosal cell samples will be collected according to work instructions detailed in the site-specific manual. The samples will be tested for SARS-CoV-2 virus using PCR tests.

7.2.6. Pregnancy Test

Serum pregnancy tests (beta human chorionic gonadotropin [β-HCG]) will be performed at Screening. Urine pregnancy tests will be used for all other scheduled visits. 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. Urine pregnancy test results should be available immediately to site. The results must be available prior to dosing (Table 1, Table 2, and Table 3).

7.2.7. Columbia-Suicide Severity Rating Scale

The C-SSRS is a questionnaire that prospectively assesses suicidal ideation and suicidal behavior. The C-SSRS, which uses a semi-structured interview to probe subject responses, will be 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 will be administered. This version assesses suicidal ideation and suicidal behavior during the subject's lifetime and during a predefined period. At all subsequent visits, the "since last visit" version will be administered.

7.2.8. Breast Cancer Imaging Surveillance

Mammograms will be performed for female subjects only. If clinically indicated, an ultrasound or MRI may also be conducted for breast cancer imaging, per the investigator's discretion.

7.3. Adverse Events, Serious Adverse Events, and Other Safety Reporting

7.3.1. Definition of Adverse Events

An AE is an untoward medical occurrence associated with the use of a study intervention in humans, whether or not it is considered related to the study intervention. An AE can therefore be any unfavorable and unintended sign (for example, an abnormal laboratory findings), symptom, or disease in a study subject who is administered with study intervention in this study.

Below are the criteria for the events that meet and do NOT meet the definition of an AE:

Events Meeting the Adverse Event Definition

- All AEs during the course of treatment with study intervention.
- All AEs resulting from medication misuse, abuse, withdrawal, or overdose of study intervention.
- All AEs resulting from medication errors such as dispensing or administration error outside of what is described in the protocol.
- Apparent unrelated illnesses, including worsening of a preexisting illness.
- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (a fall secondary to dizziness), the medical condition (dizziness)

and the accident (fall) should be reported as 2 separate AEs. The outcome of the accident (hip fracture secondary to the fall) should be recorded in source documents.

- Abnormalities in physiological testing or physical examination findings that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test).
- Laboratory or ECG abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event.

Events NOT Meeting the Adverse Event Definition

- Laboratory abnormalities associated with a clinical event should be captured in the source documents. Laboratory abnormalities not requiring clinical intervention or further investigation will be captured as part of overall laboratory monitoring and should not be reported as AEs.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studies, unless more severe than expected for the subject's conditions (see Section 7.3.11).
- Preexisting conditions (eg, allergic rhinitis) must be noted in the eCRF at Screening but should not be reported as AEs unless the medical condition worsens, or episodes increase in frequency during the AE reporting period.
- Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as AEs unless the medical condition for which the procedure was performed meets the definition of an AE. For example, an acute appendicitis that occurs during treatment with the study drug should be reported as the AE, and the resulting appendectomy should be recorded in the source documents and eCRF. If a surgical procedure was planned prior to entry into the study, and the surgery is not performed because of a worsening of a baseline condition, this should not be reported as an AE. Note that, as described in Section 7.3.2, any hospitalization occurring as the consequence of an AE during the study period should be reported as an SAE.

Each AE is to be classified as serious or nonserious by the investigator using medical and scientific judgment.

7.3.2. Definition of Serious Adverse Event

An SAE is an untoward medical occurrence or effect at any dose, regardless of whether it is related to the study intervention, which results in one of the following:

- Results in death. This includes all deaths on treatment or within 30 days after last study intervention administration, including deaths due to disease progression. Any death occurring later than 30 days following the last dose need not be reported as an SAE unless it is a result of an event that started within the period covered by the on-study definition. The reported AE should be the event that caused the death. In addition, any AE resulting in death that occurs post the AE reporting period and that

the investigator assesses as possibly related to the study intervention should also be reported as serious.

- Is life-threatening. This refers to an event in which the subject was at risk of death at the time of the event. It does not include an event that, had it occurred in a more severe form, hypothetically might have caused death.
- Requires hospitalization or prolongation of existing hospitalization (excluding hospitalizations for administration of the study intervention, procedures required by the study protocol, or treatment-related diagnostic procedures; other planned hospitalizations; or hospitalizations related only to progression of disease). Emergency room visits that do not require admission to the hospital do not fall into this category, but the event may be serious due to another seriousness criterion.
- Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions.
- Any other medically important event that the investigator or PTC judges to be serious or which is defined as serious by the regulatory agency in the local country. These are AEs that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Medical judgment should be exercised in deciding whether an AE is serious based on the above definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.
- A pregnancy resulting in spontaneous abortion, stillbirth, neonatal death, or congenital anomaly (including that in an aborted fetus).

Note: Hospitalization for social circumstances without any other accompanying SAE should not be reported as an SAE.

7.3.3. Adverse Events Reporting

AEs will be reported by the subjects (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible to pursue and obtain adequate information both to determine the outcome and to assess whether the event meets the criteria for classification as an SAE or caused the subject to discontinue the study intervention (see Section 6.1).

Each subject/parent(s)/legal guardian will be questioned about the occurrence of AEs in a nonleading manner.

In addition, the investigator may be requested by the PTC Pharmacovigilance Department to obtain specific follow-up information in an expedited fashion.

All AEs are to be recorded in the source documents and on the eCRF using concise medical terminology; whenever possible, terms contained in the Medical Dictionary for Regulatory

Activities (MedDRA) should be employed. In addition, the following information should be recorded:

- Indication of whether the event is serious or nonserious (see Section 7.3.2)
- Relationship to study intervention (see Section 7.3.5)
- Severity of the event (see Section 7.3.5)
- Onset date
- Resolution date, or date of death
- Action taken
- Outcome of the event

Table 6 below summarizes the investigator site requirements for recording AEs on the eCRF and for reporting SAEs, adverse events of special interest (AESI) on the SAE Report Form to the PTC Pharmacovigilance Department.

Table 6: Investigator Site Requirements for Recording/Reporting Adverse Events

Event	Recorded on the eCRF	Reported to the PTC Pharmacovigilance Department Within 24 Hours of Awareness
Serious AE	All	All
Nonserious AE	All	None
Other, eg, exposure to the study intervention during pregnancy or breastfeeding	All (regardless of whether associated with an AE)	All (regardless of whether associated with an AE)
AESI	Not applicable	Not applicable

Abbreviations: AE, adverse event, AESI, adverse event of special interest, eCRF, electronic care report form

7.3.4. Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

The AE (both serious and nonserious) collection and reporting period begins with the signing of the ICF and continues until 30 days after the last dose of study medication. All AEs during this time period must be recorded, whether or not the event is considered drug related.

For subjects who are screen failures, the active collection period for AEs ends when screen failure status is determined. Any SAEs occurring during screening procedures, should have the appropriate completed causality assessment (ie, “not related” to study intervention) section of the SAE Report Form completed.

If the subject withdraws from the study and also withdraws consent for the collection of future information, the active collection period for AEs ends when consent is withdrawn.

Investigators are not obligated to actively seek information on AEs or SAEs after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention, the investigator must promptly notify PTC Pharmacovigilance Department via the SAE Report Form.

7.3.4.1. Serious Adverse Event Reporting

All SAEs (both initial and follow-up) should be reported via the SAE Report Form to the PTC Pharmacovigilance Department immediately and under no circumstance should this exceed 24 hours from awareness. In addition, the AE portion of the eCRF must also be completed.

The SAE Report Form must be signed by the investigator; however, if the investigator is unable to sign at the time of the event or within 24 hours, the form should be signed by the clinical staff member reporting the SAE (eg, the study coordinator). The SAE Report Form must be faxed or emailed to the PTC Pharmacovigilance Department and to the site IRB/IEC (if required by local regulations) within 24 hours. The investigator's signature must be obtained as soon as possible after the report is submitted and the report revised if necessary, and submitted to the PTC Pharmacovigilance Department. The investigator will maintain a copy of the SAE Report Form on file at the study site.

Follow-up information to the SAE should be clearly documented as "follow-up" in the SAE Report Form and must also be faxed or emailed to the same party within the same timelines. All follow-up SAE Report Forms for the event must be signed by the investigator as per instructions above. Any source documents (eg, progress notes, nurses' notes, laboratory and diagnostic test results, discharge summaries) provided to the PTC Pharmacovigilance Department should be redacted so that the subject's name, address, and other personal identity information are obscured. Only the subject's study number and initials are to be provided (in regions where the provision of such information is permitted). The information in the AE portion of the eCRF and the SAE Report Form(s) must match or be reconciled. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same AE term should be used on both forms.

The PTC Pharmacovigilance Department contact information for reporting SAEs is provided below. This information is also provided in the SAE Report Form:

PTC Pharmacovigilance Department
Email: Pharmacovigilance@ptcbio.com
SAE FAX Line: +1-908-325-0355

7.3.5. Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

7.3.5.1. Assessments of Severity of Adverse Events

The severity of AEs will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. For each episode, the highest severity grade attained should be reported.

The investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to 1 of the following categories (Table 7).

Table 7: Grading of Adverse Events

Grade	Adjective	Description
1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Minimal, local, or noninvasive intervention indicated, limiting age appropriate instrumental Activities of Daily Living (ADL). Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling, limiting self-care ADL. Self-care ADL refers to bathing, dressing, and undressing, feeding self, using the toilet, taking medications, and not bedridden.
4	Life-threatening	Sign or symptom results in urgent intervention.
5	Fatal	Sign or symptom results in death.

7.3.5.2. Describing Adverse Event Relationship to Study Intervention

The investigator should provide an assessment of the relationship of the AE/SAE to the study intervention and each occurrence of each AE/SAE outlined in Table 8. The investigator will use clinical judgment to determine the relationship.

The investigator will also consult the IB and/or product information, for marketed products, in his/her assessment.

For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations in which an AE/SAE has occurred, and the investigator has minimal information to include in the initial SAE Report Form to the PTC Pharmacovigilance Department. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE Report Form to the PTC Pharmacovigilance Department.

The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product.

- If no valid reason exists for suggesting a relationship, then the AE should be classified as "unrelated." If a relationship cannot be excluded but there is a clear alternate cause that is more likely to have caused the AE than the study intervention, the AE should be considered "unlikely related."
- If there is any valid reason, even if undetermined, for suspecting a possible cause-and-effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered "possibly or probably related."

If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable," the event will be considered related to the investigational product for the purposes of expedited regulatory reporting. An assessment of "unlikely related" will be considered unrelated for regulatory reporting purposes.

The investigator may change his/her opinion of causality in light of follow-up information and must send an SAE follow-up report with the updated causality assessment.

The causality assessment is 1 of the criteria used when determining regulatory reporting requirements (Table 8).

Table 8: Relationship of Study Intervention to Adverse Event

Relationship	Description
Probably	A clinical event in which a relationship to the study drug seems probable because of such factors as consistency with known effects of the drug; a clear temporal association with the use of the drug; improvement upon withdrawal of the drug; recurrence upon rechallenge with the drug; lack of alternative explanations for the event.
Possible	A clinical event occurring coincident with administration of the study intervention, and which may or may not be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal or rechallenge may be lacking.
Unlikely	A clinical event with a temporal relationship to the study intervention exposure that does not preclude causality but for which there is a clear alternate cause that is more likely to have caused the adverse event than the study intervention. Such alternatives include a concomitantly administered drug, the subject's disease state, other medical conditions, or environmental factors.
Unrelated	A clinical event for which a relationship to the study intervention seems improbable because of factors such as inconsistency with known effects of the study intervention, lack of a temporal association with study drug administration, lack of association of the event with study intervention withdrawal or rechallenge, and/or presence of alternative explanations for the event. Alternative explanations might include a known relationship of the adverse event to a concomitant drug, medical history of a similar event, the subject's disease state, other medical conditions, or environmental factors.

7.3.6. Treatment of Adverse Events and Serious Adverse Events

Treatment of AEs and SAEs will be symptom-based and at the discretion of the investigator.

7.3.7. Follow-Up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 6.3).

All AEs should be followed up by the investigator until they are resolved or the investigator assesses them as chronic or stable. The investigator should consider protocol guidelines and use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events. In the event of additional investigations, the PTC Pharmacovigilance Department should be informed via email or fax.

A subject withdrawn from the study because of an AE must be followed by the investigator until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. Follow-up may need to continue after the subject has discontinued from the study, and additional investigations may be requested by the medical monitoring team.

The investigator must submit any updated SAE information to the PTC Pharmacovigilance Department within 24 hours of receipt of the information.

7.3.8. Regulatory Reporting Requirements for Serious Adverse Events

Prompt notification by the investigator to PTC of an SAE is essential so that legal obligations and ethical responsibilities toward the safety of subjects and the safety of a study intervention under clinical investigation are met.

As the sponsor of the study, PTC has a legal responsibility to notify the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. PTC will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, institutional review boards (IRBs)/independent ethics committees (IECs), and investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives SUSAR or other safety-specific information (eg, summary or listing of SAEs) from PTC will review and then file it along with the reference document and will notify the IRB/IEC, if appropriate according to local requirements.

7.3.9. PTC Expedited Reporting of SUSARs to EudraVigilance

In the EU, as per requirements of EU Clinical Trials Regulation No. 536/2014 of the European Parliament and of the Council of 16 April 2014 on Clinical Trials on Medicinal Products for Human Use, and Repealing Directive 2001/20/EC, SUSARs should be submitted to the EudraVigilance database. Additional direct submissions from the Sponsor to Ethics Committees and National Competent Authorities are not required.

As per the EU Clinical Trials Regulation, the Sponsor shall notify the Member States concerned of all findings which affect the benefit-risk balance of the medicinal product or clinical trial, and any urgent safety measures (USMs) taken. The Sponsor will submit findings that affect the benefit-risk balance or any USM via the Clinical Trials Information System (CTIS).

7.3.10. Exposure During Pregnancy or Breastfeeding

Exposure to the study intervention under study during pregnancy or breastfeeding are reportable to the PTC Pharmacovigilance Department within 24 hours of investigator awareness.

7.3.10.1. Exposure During Pregnancy

PTC Pharmacovigilance Department must be notified in the event that a female subject in the study, or a female partner of a male subject in the study, becomes pregnant on-study or within 90 days of the last dose of the study intervention on an Investigational and Marketed Products Pregnancy Report Form (hereafter referred to as Pregnancy Report Form) (see study manual for details). This must be reported whether or not an AE has occurred and within 24 hours of awareness of the pregnancy. The information submitted should include the anticipated date of birth or pregnancy termination.

Written consent is required prior to collecting and reporting any information on a female partner of a male subject in the study.

In any of the 4 situations listed below, the subject will be provided with a Pregnancy/Pregnant Partner Data Release Form to request their consent to follow the progress of the pregnancy and the birth and the health of their child.

- Subject becomes pregnant while participating in the study.
- Female partner of a male subject participating in the study becomes pregnant.
- Subject becomes pregnant up to 30 days after completion of the study.
- Female partner of a male subject participating in the study becomes pregnant up to 90 days after the partner completed the study.

Because the risk to an unborn child is unknown, the subject should be asked to sign the Pregnancy/Pregnant Partner Release Form. However, signing this form is voluntary; it is up to the subjects to decide whether to agree to the collection of this information or not. Upon signing, the subject's and the child's medical records relating to the pregnancy and delivery, and the health of the child, will be reviewed for up to 1 year of age.

If possible, the investigator should follow the subject, or the pregnant female partner of a male subject, until completion of the pregnancy and notify the PTC medical monitor of the outcome within 5 days or as specified below. The investigator must provide this information as a follow-up to the initial Pregnancy Report Form via the Pregnancy Outcome Section (see the study manual for details).

If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (ie, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly [including that in an aborted fetus]), the investigator should follow the procedures for reporting SAEs (ie, report the event to PTC Pharmacovigilance Department and follow-up by completion of appropriate AE eCRFs and submission of the SAE Report Form).

All information collected with regard to the pregnancy, delivery of the child, and the health of the child is confidential to the limit allowed by law. These data will be coded to hide the subject's identity and the identity of the child. In particular, the subject's name and the child's name will not be reproduced on any other paper or electronic document. These data will not be disclosed voluntarily by PTC. However, regulatory agencies may have to examine these data to ensure that the study is done properly.

Of note, if the pregnant female partner of a male study subject participating in the study does not wish to provide such information, this will not prevent the partner from continuing with the study.

Any female subject who becomes pregnant while participating in the study will discontinue study intervention/withdrawn or be excluded from the study (see Section 4.3).

7.3.10.2. Exposure During Breastfeeding

An exposure during breastfeeding occurs if:

- A female subject is found to be breastfeeding while receiving or up to 30 days after discontinuing study intervention.

- A female is found to be breastfeeding while being exposed or having been exposed to study intervention (ie, environmental exposure). An example of environmental exposure during breastfeeding is a female family member or healthcare provider who reports that she is breastfeeding after having been exposed to the study intervention by inhalation or skin contact.

The investigator must report exposure during breastfeeding to the PTC Pharmacovigilance Department within 24 hours of the investigator's awareness, irrespective of whether an SAE has occurred. The information must be reported using the Pregnancy Report Form. When exposure during breastfeeding occurs in the setting of environmental exposure, the exposure information does not pertain to the subject enrolled in the study, so the information is not recorded on an eCRF. However, a copy of the completed Pregnancy Report Form is maintained in the investigator site file. An exposure during breastfeeding report is not created when a PTC drug specifically approved for use in breastfeeding women (eg, vitamins) is administered in accordance with authorized use. However, if the infant experiences an SAE associated with such a drug, the SAE is reported together with the exposure during breastfeeding via SAE Report Form.

7.3.11. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

Not applicable.

7.3.11.1. Progression of Underlying Disease

If the progression of the underlying disease is greater than that which would normally be expected, or if the investigator considers that there may be a causal relationship between the investigational medicinal product or protocol design/procedures and the disease progression, then it must be reported to the PTC Pharmacovigilance Department within 24 hours of awareness.

7.3.11.2. Abnormal Findings Associated with the Disease Being Studied

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, should not be reported as AEs or SAEs.

7.3.12. Contraceptive Guidance

7.3.12.1. Male Participant Reproductive Inclusion Criteria

Male subjects are eligible to participate if they agree to the following requirements during the intervention period and for at least 90 days after the last dose of study intervention, which corresponds to the time needed to eliminate reproductive safety risk of the study intervention(s):

- Refrain from donating sperm.

PLUS, either:

- Be abstinent from heterosexual intercourse with a female of childbearing potential as their preferred and usual lifestyle (abstinent on a long-term and persistent basis) and agree to remain abstinent.

OR

- Must agree to use a male condom when engaging in any activity that allows for passage of ejaculate to another person.
- In addition to male condom use, a highly effective method of contraception may be considered in WOCBP partners of male subjects (refer to the list of highly effective methods below in Section 7.3.12.4).

7.3.12.2. Female Participant Reproductive Inclusion Criteria

A female subject is eligible to participate if she is not pregnant or breastfeeding, and at least1 of the following conditions applies:

- Is not a woman of childbearing potential (WOCBP) (see definitions below in Section 7.3.12.3).

OR

- Is a WOCBP and using an acceptable contraceptive method as described below during the intervention period (for a minimum of 90 days after the last dose of study intervention). The investigator should evaluate the effectiveness of the contraceptive method in relationship to the first dose of study intervention. The investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy.

7.3.12.3. Women of Childbearing Potential

Women of childbearing potential includes any female who is fertile, following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy.

Postmenopausal is defined as no menses ≥12 months with or without an alternative medical cause. In addition, a high follicle-stimulating hormone level in the postmenopausal range must be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy.

7.3.12.4. Contraception Methods

Below are the general contraceptive methods (which are both highly and non-highly effective) use by men or women should be consistent with local availability/regulations regarding the use of contraceptive methods for those participating in clinical trials.

1. Intrauterine device.
2. Intrauterine hormone-releasing system.

3. Bilateral tubal occlusion.
4. Vasectomized partner:
    - Vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. The spermatogenesis cycle is approximately 90 days.
5. Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Intravaginal
    - Transdermal
6. Progestogen-only hormone contraception associated with inhibition of ovulation:
    - Oral
    - Injectable
    - Implantable
7. Sexual abstinence:
    - Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject

7.3.13. Adverse Events of Special Interest

There are currently no identified AESI for PTC857.

7.3.14. Medication Errors

Medication errors may result from the administration or consumption of the study intervention by the wrong subject, or at the wrong time, or at the wrong dosage strength.

Table 9: Investigator Site Requirements for Recording/Reporting Medication Errors

Event	Recorded on the eCRF	Reported to the PTC Pharmacovigilance Department Within 24 Hours of Awareness
Medication errors	All (regardless of whether associated with an AE)	Only if associated with an SAE

Abbreviations: AE, adverse event; eCRF, electronic case report form; SAE, serious adverse event

Medication errors include the following:

- Medication errors involving subject exposure to the study intervention

- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the study subject
- The administration of expired study intervention
- The administration of an incorrect study intervention
- The administration of an incorrect dosage
- The administration of study intervention that has undergone temperature excursion from the specified storage range, unless it is determined by PTC that the study intervention under question is acceptable for use.

Such medication errors occurring to a study subject are to be captured in source and eCRF as applicable.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error is recorded on source and, if applicable, any associated AE(s), serious and nonserious, are recorded on the AE page of the eCRF. Medication errors should be reported to the PTC Pharmacovigilance Department within 24 hours on a SAE Report Form only when associated with an SAE.

7.3.15. Safety Oversight

An independent DSMB will be utilized during the study. The DSMB will closely monitor potential emerging safety signals in all subjects in the study throughout their study participation, including the planned transition without interruption in treatment from Part A to Part B of the study.

8. STATISTICAL CONSIDERATOINS

A Statistical Analysis Plan (SAP) will be prepared before database lock.

8.1. Statistical Hypotheses

For the primary efficacy endpoint, the null hypothesis of this study is that the subject ranks based on the composite survival and change from baseline in ALSFRS-R after the 24-week Treatment Period are the same in the PTC857 group and in the placebo group versus the alternative that they are different.

The primary efficacy endpoint will be analyzed on the ITT1 Analysis Set at the significance level of 0.05 (2-sided). If $p<0.05$, then the study will be declared positive.

8.2. Sample Size Determination

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 2.5 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. Data from the 2 recently concluded clinical studies in ALS reported overall death rates of 5% and 0% (Edaravone (MCl-186) ALS 19 Study Writing Group 2017, Paganoni 2020). In addition, the edaravone study reported a standard deviation of 5.3 points for the ALSFRS-R score at Week 24, among treated subjects. 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 sampling for missing data is not planned.

8.3. Population for Analyses

Intent-to-Treat 1 Analysis Set: 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. The ITT1 Analysis Set will be used for analysis of the primary endpoint.

Intent-to-Treat 2 Analysis Set: 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.

Per Protocol Analysis 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. The criteria for inclusion in the PP Analysis Set will be finalized prior to study unblinding and detailed in the SAP.

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.

PK Analysis Set: All subjects in the ITT2 Analysis Set who have at least 1 measurable plasma or CSF PTC857 concentration will be included in the PK Analysis Set.

8.4. Statistical Analyses

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in the SAP. The analysis of the data for the Treatment Period (Part A) (ie, up to the Week 24 Visit) will be produced after the lock of database for Part A. The LTE Period (Part B) of the study will continue for all subjects participating in that part, and results from Part B will be reported separately. The Continued LTE Period (Part C) of the study will continue for all subjects participating in that part, and the results from Part C will also be reported separately.

8.4.1. Primary Efficacy Endpoint

For the primary efficacy endpoint, subject ranks based on the combined assessment of function and survival (CAFS) ranking method, will be analyzed when all subjects in the ITT1 Analysis Set finish the Treatment Period (Part A). This analysis will be performed using an analysis of covariance (ANCOVA) model following multiple imputation. Subjects who discontinue from the study prior to Week 24 will have their Week 24 ALSFRS-R score imputed using multiple imputation. The ANCOVA model will include the following covariates: treatment arm (PTC857 and placebo), baseline ALSFRS-R score, 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 standard-of-care exposure with rank scores as the response variable. A p value for the difference will be presented.

The analysis of the primary efficacy endpoint will occur when the Part A database is locked.

Sensitivity and subgroup analyses of the primary efficacy endpoint will be described in the SAP.

8.4.2. Secondary Efficacy Endpoints (Part A)

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 model will include fixed effects for treatment (PTC857 and placebo), baseline ALSFRS-R value, time of collection (actual week at the visit as a categorical variable), time-by-treatment interaction, time-by-baseline ALSFRS-R interaction, the amount of change in the subject's ALSFRS-R score during the Screening Period (<1, 1-2, and 3-4 points total loss), standard-of-care exposure, TRICALS risk profile, and TRICALS risk profile by time interaction as covariates. The unstructured covariance matrix (UN) will be used in the model. 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 the ITT1 and ITT2 analysis sets.

Analysis of other secondary endpoints and sensitivity analyses will be specified in the SAP.

8.4.3. Long-Term Extension Period Efficacy Endpoints (Part B)

The analysis details for the LTE Period will be included in the SAP.

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

The analysis details for the Continued LTE Period will be included in the SAP.

8.4.5. Safety Analyses

Safety is measured by the severity and number of TEAEs and TESAEs, change from baseline in clinical laboratory tests, physical examination, vital signs, and 12-lead ECGs during the Treatment Period.

Baseline is defined as the last value measured prior to first dose intake (vital signs, 12-lead ECG, laboratory parameters, C-SSRS), unless otherwise specified.

The following have been defined as parameters regarding safety and tolerability:

- Change from baseline to each scheduled timepoint up to EOS for vital signs
- Change from baseline to each scheduled timepoint up to EOS for 12-lead ECG parameters
- Change from baseline to each scheduled timepoint up to EOS for clinical laboratory tests
- Changes from baseline in C-SSRS scores
- Treatment-emergent AEs up to EOS
- Treatment-emergent AEs leading to premature discontinuation of study drug
- Treatment-emergent SAEs up to EOS

All safety data will be populated in the individual eCRFs. Safety data, including dosing dates and times, will be listed by subject.

All AEs and SAEs will be coded using the most current version of MedDRA available at PTC and summarized by treatment for the number of subjects reporting the TEAEs/TESAEs and the number of TEAEs reported.

The TEAEs/TESAEs are tabulated by System Organ Class (SOC) and individual Preferred Terms within each SOC by treatment group. The number and percentage of subjects who experienced AEs coded with the same Preferred Term and SOC will be summarized by treatment group (in descending order according to the incidence in the investigational study drug group). Adverse events will also be tabulated by severity and by relationship to study drug. Summary tables will be accompanied by individual subject listings broken down by treatment group, including predose events.

Reasons for death will only be listed. Reasons for premature discontinuation of study drug will be listed and summarized by frequency tables.

Safety data, including 12-lead ECGs, vital signs assessments, and clinical laboratory results, will be summarized at each timepoint using mean, median, standard deviation, minimum, maximum, number of available observations, and change from baseline. Individual subject listings of 12-lead ECG data, vital signs data, laboratory measurements, and C-SSRS scores will be provided.

Standard numeric laboratory parameters are presented in the units supplied. If needed, a conversion will be made to standard units.

Quantitative safety data as well as the difference from baseline, when appropriate, will be summarized using the appropriate descriptive statistics.

Concomitant medications will be listed by subject and coded using the most current version of World Health Organization (WHO) drug dictionary available at PTC.

8.4.6. Pharmacokinetic Analyses

The plasma PTC857, riluzole, edaravone, and/or sodium phenylbutyrate/taurursodiol concentrations, the PK parameters, and CSF exposure listed in Section 7.1.8.4 and Section 7.1.8.5 will be summarized using appropriate descriptive statistics, which will be fully outlined in the PK SAP.

8.5. Interim Analysis

No interim analysis is planned for this study.

9. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

In accordance with 21 Code of Federal Regulations Part 312.56 and/or relevant ICH guidelines, PTC or a designee will periodically inspect all eCRFs, study documents, research facilities, and clinical laboratory facilities associated with this study at mutually convenient times, before, during, and after completion of the study. As required by applicable regulations (FDA 2020), the monitoring visits provide PTC with the opportunity to evaluate the progress of the study; verify the accuracy and completeness of data in the eCRFs; ensure that all protocol requirements, relevant regulations, and investigator's obligations are being fulfilled; and resolve any inconsistencies in the study records. This includes inspection of all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the subjects in this study. The names and identities of all research subjects will be kept in strict confidence and will not appear on eCRFs or other records provided to or retained by PTC. The investigator/institution guarantees direct access to source documents by PTC or a designee and appropriate regulatory authorities.

It is important that the investigator and relevant institutional personnel are available during the monitoring visits and possible audits, or inspections and that sufficient time is devoted to the process.

9.1. Study Monitoring

Before a potential investigational site can be selected to conduct the study, a representative of PTC or its designee will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence and the responsibilities of PTC or its representatives. This will be documented in a Clinical Study Agreement between PTC and the investigator

During the study, a monitor from PTC or representative will have regular contacts with the investigational site for the following:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the case report forms, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the case report forms with the patient's medical records at the hospital or practice and other records relevant to the study. This will require direct access to all original records for each patient (eg, clinic charts)
- Record and report any protocol deviations not previously sent to PTC

- Confirm AEs and SAEs have been properly documented on eCRFs and confirm any SAEs have been forwarded to PTC and those SAEs that met criteria for reporting have been forwarded to the IRB/IEC

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

9.2. Audits and Inspections

PTC may conduct audits of clinical research activities in accordance with internal Standard Operating Procedures to evaluate compliance with the principles of Good Clinical Practice (GCP) and ICH-related guidelines.

Health authorities may also wish to conduct an inspection (during the study or after its completion). Should an inspection be requested by health authorities, the investigator must inform PTC immediately that such request has been made.

The investigator will permit such audits by PTC or health authorities and facilitate them by providing access to the relevant source documents.

10. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, PTC, PTC's representatives, a regulatory authority, IEC, and/or IRB may conduct a quality assurance audit. Reasons for quality assurance audit may include, but are not limited to, random selection, geographic proximity, suspected GCP violation, high enrolling site, recurring protocol deviations, etc. The purpose of an audit or inspection by PTC is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. The investigator should contact PTC immediately if contacted by a regulatory agency about an inspection.

10.1. 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 deviations are any deviations that impact subject eligibility (ie, protocol inclusion/exclusion violations), subject safety, or a subject's ability to continue in the study.

At the outset of the study, a process for defining and handling protocol deviations will be established with the contract research organization (CRO). This will include determining which deviations will be designated major, thus requiring immediate notification to the medical monitor and PTC. Prospective deviations (eg, protocol waivers) are prohibited by PTC policy. The investigator is responsible for seeing that any known protocol deviations are recorded as agreed.

11. ETHICS

11.1. Ethics Review

The PI must obtain IRB/IEC approval for the investigation. Initial IRB/IEC approval and all materials approved by the IRB/IEC for this study, including the patient consent form and recruitment materials, must be maintained by the investigator and made available for inspection.

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. The investigator must submit written approval to PTC before he or she can enroll any subject into the study.

The PI is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The PI is also responsible for providing the IRB or IEC with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. PTC will provide this information to the PI.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

11.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP and applicable regulatory requirements, including EU Regulation No. 536/2014.

11.3. Written Informed Consent

By signing the protocol, the investigator assures that informed consent will be obtained from each subject/subject's designee (legally authorized representative or caregiver) prior to study entry and that the informed consent will be obtained in accordance with current regulations.

The investigator or sub-investigator will give each subject full and adequate verbal and written information regarding the objectives and procedures of the study and the possible risks involved. An informed consent document will be provided to each subject or designee in a language in which they are fluent. This information must be provided to the subject prior to undertaking any study-related procedure. Adequate time should be provided for the subject or designee to read the informed consent, to understand the risks and benefits of participating in the study, and to ask any questions that the subject may have about the study. The subject's signature on the ICF should be obtained at the investigator site in the presence of the investigator or a qualified representative (eg, sub-investigator).

The original signed ICFs will be retained by the investigator with the study records. The written subject information must not be changed without prior approval by PTC and the IRB/IEC.

Due to the nature of ALS disease progression, it is anticipated that subjects who may not be able to provide ongoing written informed consent for the duration of the study, but could provide ongoing nonwritten informed consent, may be enrolled. Hence, nonwritten consent, which may

require the presence of a witness, may be provided in accordance with country-specific legislations as applicable.

In addition, disease progression may make it difficult to attend on-site visits; hence, subjects may provide informed consent remotely, in accordance with site process and local requirements/regulations. The study site should provide the consent form in hard copy to the subject by post or delivered by a home healthcare professional. The subject's written consent would be gathered on the consent form and collected by the home healthcare professional at a home visit.

12. DATA HANDLING AND RECORDKEEPING

To enable evaluations and/or audits from regulatory authorities or PTC, the investigator agrees to keep accurate and complete records, including the identity of all participating subjects (sufficient information to link eCRFs and clinic records/source documents), all original signed ICFs, electronic copies (ie, CD-ROM, USB, etc) or paper copies of the data that have been captured in the EDC for each subject (eCRFs), and detailed records of study drug disposition.

The investigator agrees to keep all information provided by PTC in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC. Study documents provided by PTC will be stored appropriately to ensure their confidentiality. The information provided by PTC to the investigator may not be disclosed to others without direct written authorization from PTC, except to the extent necessary to obtain informed consent from subjects who wish to participate in the study.

All records and documents pertaining to the study will be maintained by the investigator until notification is received from PTC that the records no longer need to be retained. The investigator must obtain written permission from PTC before disposing of any records. The investigator will promptly notify PTC in the event of accidental loss or destruction of any study records. If the investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to PTC as applicable.

12.1. Inspection of Records

PTC will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, Drug Accountability Records, subject charts and study source documents, and other records relative to study conduct.

12.2. Retention of Records

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents will be classified into 2 different categories: investigator’s file and subject clinical source documents.

The investigator’s file will contain the protocol/amendments, eCRFs, IEC/IRB and Health Authority approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, screening and enrollment logs, and other appropriate documents/correspondence as per ICH/GCP and local regulations.

Subject clinical source documents include, but are not limited to, subject hospital/clinic records, physician’s and nurse’s notes, appointment book, original laboratory, 12-lead ECG, pathology and special assessment reports, consultant letters, etc.

These 2 categories of documents must be kept on file by the investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval or 25 years, whichever is longest). No study document should be destroyed without prior written approval from PTC. Should the investigator wish to assign the study records to another party, or move them to another location, PTC must be notified in advance.

When source documents are required for the continued care of the subject, appropriate copies should be made for storing outside of the site.

The handling of personal data will comply with local regulations.

12.3. Future Use of Stored Specimens and Data

As part of the current study, blood, urine, and CSF will be collected. Sample processing will be performed by a laboratory under the direction of PTC. Processing, analysis, and storage will be performed at a secure laboratory facility to protect the validity of the data and maintain subject privacy. Samples will only be used for the purposes described in this protocol. Laboratories contracted to perform the analysis on behalf of PTC will not retain rights to the samples beyond those necessary to perform the specified analysis and will not transfer or sell those samples. PTC will not sell the samples to a third party.

PTC may store biospecimen for up to 5 years after the end of the study, in accordance with local regulations, to achieve study objectives. Additionally, with the subject's consent, samples may be used for further research by PTC to contribute to the understanding of ALS. A section within the ICF will address the use of remaining samples for optional exploratory research.

Further research may include the biomarkers of neurodegeneration, synaptic function, inflammation, and metabolism/oxidative stress, if new assays are developed or validated and/or if more robust longitudinal and cross-sectional data are available.

Subjects may request their samples be destroyed at any time. Samples collected while the subject was participating under consent of the study will be stored and analyzed, if necessary, in order to maintain the ability to analyze any samples if requested by a regulatory authority, or for any potential safety and/or efficacy reasons.

At the end of the storage period, samples will be destroyed. Samples may be stored longer if a health authority (or medicinal product approval agency) has active questions about the study. In this special circumstance, the samples will be stored until the questions have been adequately addressed. No subject-identifying information (eg, initials, date of birth, government identifying number) will be associated with any of the biologic samples. All samples will be single coded. PTC will take steps to ensure that data are protected accordingly, and confidentiality is maintained as far as possible.

Data from subjects enrolled in this study may be analyzed worldwide, regardless of location of collection. PTC and its representatives and agents may share coded data with persons and organizations involved in the conduct or oversight of this research. These include the following:

- CROs retained by PTC
- IECs or IRBs that have responsibility for this research study
- National regulatory authorities or equivalent government agencies

At the end of the analysis, results may be presented in a final report that can include part or all of the coded data in listing or summary format. Other publication (eg, in peer-reviewed scientific journals) or public presentation of the study results will only include summaries of the population in the study, and no identified individual results will be disclosed. Given the research nature of the laboratory analysis, it will not be possible to return individual data to subjects. The results that may be generated are not currently anticipated to have clinical relevance to the patients or their family members. Therefore, these results will not be disclosed to the patients or their physicians.

13. PUBLICATION POLICY

The information developed during the conduct of this clinical study is considered confidential by PTC. This information may be disclosed as deemed necessary by PTC.

PTC intends that the data from this study will be presented and published. The PTC staff under the direction of the PTC Chief Development Officer or designee in collaboration with the investigator will be responsible for writing presentations and manuscripts for publication. Investigators will not be allowed to publish or present the data from this study without prior agreement with PTC.

The investigator is obliged to provide PTC with complete test results and all data derived by the investigator from the study. During the study, only PTC may make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the Clinical Study Site Agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of PTC.

PTC may publish any data and information from the study (including data and information generated by the investigator) jointly with the investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the Clinical Study Site Agreement. In the event of any discrepancy between the protocol and the Clinical Study Site Agreement, the Clinical Study Site Agreement will prevail.

Data from all sites participating in the study will be pooled and analyzed by PTC or PTC's designee. The first publication of the study results shall be made in conjunction with the results from other study sites as a multicenter publication. If a multicenter publication is not forthcoming within 24 months of completion of the study at all sites, the investigator may publish or present the results generated at his or her site.

The investigator will provide PTC with a copy of any proposed publication or presentation for review and comment at least 60 days prior to such presentation or submission for publication. PTC shall inform the investigator in writing of any changes or deletions in such presentation or publication required to protect PTC's confidential and proprietary technical information and to address inaccurate data or inappropriate interpretations in the context of any pooled multicenter results. At the expiration of such 60-day period, the investigator may proceed with the presentation or submission for publication unless PTC has notified the institution or the investigator in writing that such proposed publication or presentation discloses PTC's confidential and proprietary technical information. Furthermore, upon the request of PTC, the investigator will delay the publication or presentation for an additional 90 days to permit PTC to take necessary actions to protect its intellectual property interests.

14. PROTOCOL AMENDMENT HISTORY

Version 1.0: 19 January 2022

Version 2.0: 14 March 2022

Version 3.0: 13 July 2022

Version 4.0: 31 October 2022

Version 5.0: 08 September 2023

Version 6.0: 14 June 2024

14.1. Version 6.0: 14 June 2024

The overall reason for Version 6.0 of the protocol was to revise the list of objectives and endpoints and update the statistical methodology for the study.

Section #	Description of Change	Brief Rationale
Protocol	The version number and date were updated throughout. Editorial and administrative revisions (eg, typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis, study design figure, and Schedule of Assessments were updated to be consistent with the changes in the protocol. The EU CT number was added and PTC's address was updated throughout.	Update
Section 1.2 Table 4	Added details on the flavored formulation of PTC857.	Update
Section 1.4.1	Added details on the completed embryo-fetal development and fertility and early embryonic development studies.	Update
Section 1.4.2	Added details on the 3 additional completed Phase 1 studies.	Update
Section 1.5.2 Section 11.3	Added details on nonwritten consent and remote consent.	Update
Section 2.1.2 Section 2.1.3 Section 2.2.2 Section 2.2.3 Section 2.2.4	Neuropsychological function was moved to be an exploratory objective and endpoint rather than secondary. An objective for evaluating the efficacy in reducing disease progression in the population with any baseline rate of disease function was added. Revised the change from baseline in respiratory function to be change from baseline in slow vital capacity. Neurofilament light chain activity was added as a secondary objective and endpoint. Exposure-response relationships was added as an exploratory endpoint.	Update
Section 2.2.1	The wording of the primary endpoint was updated to match the modified statistical analysis method.	Update
Table 1 Table 2	Added a note that for subjects who early terminate from the study and consent to continued follow-up, the investigator should continue to follow-up for vital status and conduct the ALSFRS-R assessment, as per local requirements/regulations.	Update

Section #	Description of Change	Brief Rationale
	Specified that urine creatinine should be tested when urine p75NTR is sampled. Specified that visits indicated as in-person only may be conducted remotely following discussion and indicated the minimum required assessments at such visits. Added a note that the ALSFRS-R must be performed by a certified rater.	
Table 3	Added a note that for subjects who early terminate from the study and consent to continued follow-up, the investigator should continue to follow-up for vital status and conduct the ALSFRS-R assessment, as per local requirements/regulations. Added weight and BMI, 12-lead ECG, vital signs, physical examinations, and clinical laboratory tests to the Schedule of Assessments for Part C. Reduced mammograms to occur yearly. Updated matching table footnotes. Specified that visits indicated as in-person only may be conducted remotely following discussion and indicated the minimum required assessments at such visits. Added a note that the ALSFRS-R must be performed by a certified rater.	Update
Section 3.3 Section 3.4 Section 8.2	Update the sample size justification in accordance with the updated primary endpoint statistical methodology.	Update
Section 4.2 Section 4.6	Specified that if a subject is discontinued from standard-of-care therapy due to removal of the product from the market, it will not be considered a protocol deviation.	Update
Section 7.1.7	Removed CSF hydroxynonenal panel from biomarker assessments.	Update
Section 7.1.8.3	Added to clarify that evaluation of PK in CSF samples will only be performed for PTC857.	Update
Section 7.2.1	Removed the statement that vital signs will be performed within approximately 15 minutes of the scheduled timepoint.	Update
Section 7.3.9	Added a section on PTC expedited reporting of SUSARs to EudraVigilance.	Update
Section 8.3	Revised the definition of the ITT1 Analysis Set.	Clarification
Section 8.1 Section 8.4.1	Revised the primary efficacy endpoint analysis methodology.	Update
Section 8.4.2	Added statistical methodology for the change from baseline in ALSFRS-R at Week 24.	Update
Section 11.2	Added reference to EU Regulation No. 536/2014	Update
Section 12.3	Clarified storage of samples to be “biospecimen.”	Clarification

14.2. Version 5.0: 08 September 2023

The overall reason for Version 5.0 of the protocol was to include Part C of the study.

Section #	Description of Change	Brief Rationale
Protocol	The version number and date were updated throughout. Editorial and administrative revisions (eg, typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity.	Update

Section #	Description of Change	Brief Rationale
	The synopsis, study design figure, and Schedule of Assessments were updated to be consistent with the changes in the protocol.	
Section 1.4.1	Results of the in vivo phototoxicity study of PTC857 were added. Subsequently, measures to protect against sun and ultraviolet light are not necessary with PTC857 treatment, and these recommendations were removed.	Update
Section 1.4.2 Section 16.1	Results from the completed food effect and drug-drug interaction Phase 1 studies of PTC857 were added.	Update
Section 2.1.3 Section 2.2.3 Section 2.2.4	Added objectives and endpoints for quality of life as assessed by EQ-5D-5L.	Update
Section 2.1.5 Section 2.2.5	Objectives and endpoints for Part C of the study were added.	Update
Section 2.2.4	Endpoints for Part B of the study were modified to provide a concrete timepoint for analysis.	Update
Section 3.2 Section 5.5	Specified that the investigator must evaluated the individual subject's standard-of-care therapy if they are not receiving any at the time of entering the Screening Period.	Update
Section 3.2 Section 5.3.1	Modified the phrasing of the stratification factors for simplicity.	Update
Section 3.1 Section 3.2 Table 3 Section 3.5.1 Section 3.5.3 Table 4 Section 5.3.1 Section 5.3.2 Section 6.4 Section 8.4 Section 8.4.4	Added the study design and description of Part C.	Update
Table 1	Added that Baseline Visit procedures may be performed within the 3 days before the day that study drug is first administered.	Update
Table 1 Table 2	Added that the ICF for Parts B and C should be presented to the subject prior to Day 169 of Part A and Day 365 of Part B, respectively, to ensure the subject has adequate time to make a decision on participation in Part B or Part C. Defined that the Day 169 and Day 365 visits are only considered the End of Treatment Visit if the subject does not choose to participate in Part B or Part C, respectively.	Update
Table 1	Added a visit window for the collection of CSF samples.	Update
Section 3.5.4	Added a window for the duration of the standard-of-care therapy must be stable for prior to enrollment in the study.	Update
Section 3.6	Modified the end of study definition for clarity among Parts A, B, and C.	Update
Section 4.2	Removed the body mass index inclusion criterion. Added a reference to the laboratory manual for inclusion criterion 4. Removed the inclusion criterion for willing and able to swallow study drug (oral solution) at randomization.	Update

Section #	Description of Change	Brief Rationale
	Revised the inclusion criterion on concomitant medications to require medications be stable and unchanged from 14 days prior to the start of the Screening Period, and moved the standard-of-care therapy requirements prior to the Screening Period to be a separate inclusion criterion.	
Section 4.3	Removed language stating that lumbar punctures will only be performed at select clinical sites.	Update
Section 4.6	Separated subject withdrawal criteria activities by discontinuation study treatment or completely from the study.	Update
Section 5.1.2	Added specifications around administering PTC857 with a feeding tube.	Update
Section 6.4	Added that PTC will continue to follow-up on long-term outcomes as per local regulations with the subject's consent.	Update
Section 7.1.1 Section 7.1.2 Section 7.1.3 Section 7.1.4 Section 7.1.6 Section 7.1.7 Section 7.1.8 Section 7.2 Section 7.2.1 Section 7.2.2 Section 7.2.3 Section 7.2.4 Section 7.2.7 Section 7.2.8	Removed redundant references throughout the efficacy and safety assessments that referred to the Schedule of Assessments.	Update
Section 7.1.5	Added a section for the EQ-5D-5L assessment.	Update
Section 7.2.5.1 Section 7.2.5.2 Section 7.2.5.3	Sections on hematology, blood chemistry, and urinalysis were removed to reduce redundancy with that presented in Section 7.2.5	Update
Section 8.4.2 Section 8.4.3	Removed specification in SAP for Part A and Part B as there will be a single SAP inclusive of all parts of the study.	Update
Section 16.1	Removed moderate inhibitors and inducers from Table 10 and Table 11, respectively, based on the results of the Phase 1 clinical DDI study.	Update

14.3. Version 4.0: 31 October 2022

Overall Rationale for the Amendment

The primary purpose of this amendment is to implement study design changes subsequent to agreement with health agencies and ethic committees and to incorporate changes to widen enrollment criteria for the study.

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Section #	Description of Change	Brief Rationale
Throughout	Minor editorial and document formatting revisions	Minor, therefore, have not been summarized

Section #	Description of Change	Brief Rationale
	The mITT and ITT analysis sets were renamed to ITT1 and ITT2, respectively.	Update
Section 1.4.1 Section 1.5.1	Added results of the 9-month monkey toxicity study, nonclinical phototoxicity study, and dose-range finding embryo-fetal development nonclinical studies.	Update
Section 1.5.2 Section 11.3	Expanded on background information on ALS, as well as considerations for ongoing written informed consent.	Update
Section 1.5.3	Added a summary of safety risks.	Update
Table 1 Table 2	Added a monthly urine pregnancy test. Added vital signs assessment on Days 29, 57, 113, 141, 197, and 225. Added that the Day 113 and 141 Visits are in-home to assess vital signs. Added a mammogram assessment at the Early Termination Visit. Added coagulation to the list of clinical laboratory tests. Specified that a serum pregnancy test will be performed at Screening, then all other pregnancy tests will be via urine.	Update
Table 1 Section 7.1.8.2 Section 7.1.8.5	Added blood sampling for edaravone and sodium phenylbutyrate/taurursodiol for those in the PK sub-study.	Update
Section 3.2 Section 8.4.1	Included additional stratification factors for use of edaravone and sodium phenylbutyrate/taurursodiol.	Update
Section 4.2	Updated the upper limit of age for enrollment to be 80 years. Updated the time frame for onset of the first symptoms leading to the diagnosis of ALS to be ≤24 months at the time of the initial Screening Visit.	To widen enrollment criteria
Section 4.3	Specified the criteria for hepatic insufficiency. Added a note that subjects with a contraindication to lumbar punctures may still enroll but should not undergo the lumbar punctures as listed in the Schedule of Events. Modified exclusion criterion 11 to state that the combination of edaravone and sodium phenylbutyrate/taurusodiol will be excluded. Added an exclusion criterion prohibiting the use of a non-approved form of sodium phenylbutyrate and/or taurursodiol for the treatment of ALS.	Update
Section 5.1.2	Added that subjects should be advised to limit sun and ultraviolet light exposure.	To prevent the potential for phototoxicity.
Section 5.2.2	Updated the storage of study drug.	Update
Section 5.3	Added criteria for emergency unblinding of study information.	Update
Section 5.5 Section 16.1	Added clarifications surrounding allowance of standard-of-care therapies.	Update
Section 7.1.7	Updated the biomarkers planned to be evaluated.	Update
Section 7.2.6	Specified that a serum pregnancy test will be performed at Screening and all other pregnancy tests will be via urine.	Update

Section #	Description of Change	Brief Rationale
Section 7.3	Updated the entirety of the Adverse Events, Serious Adverse Events, and Other Safety Reporting section.	For consistency with updated safety reporting language and to align with the new PTC protocol template pharmacovigilance language.
Section 12.3	Modified the duration and future use of stored specimens to be stored for up to 5 years are the end of the study, in accordance with local regulations. Samples may be used for future research with subject's consent.	Update
NA	The acceptable birth control methods appendix was deleted as the content was moved to Section 7.3.11.4.	Update

14.4. Version 3.0: 13 July 2022

Overall Rationale for the Amendment

The primary purpose of this amendment is to include additional inclusion/exclusion criteria and assessments to ensure the safety of enrolled subjects.

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Section #	Description of Change	Brief Rationale
Throughout	Minor editorial and document formatting revisions	Minor, therefore, have not been summarized
Section 1.4.1 Section 1.5.1 Section 3.5.2	Additional text was added to provide information from the 6-month rat toxicology study.	Update
Section 3.2	Specified that subjects in Part B will remain blinded to their original treatment from Part A.	Clarification
Table 1 Table 2 Section 7.2.8	Added breast cancer imaging assessments for female subjects at Screening and approximately every 6 months during the Treatment and Long-Term Extension periods.	Additional assessment included for continued safety monitoring of subjects.
	Added that subjects may go to the site for an in-person visit if an in-home visit is not possible.	Clarification
	Clarified language surround lumbar puncture requirements at the selected clinical sites.	Clarification
Section 4.2	Specified that subjects or their designee must provide written informed consent.	To allow flexibility in consent
	Added that female subjects must have a negative breast cancer screening status within 6 months of the Screening Visit or during the Screening Period.	To ensure the safety of enrolled subjects
Section 4.3	Added that female subjects or female subjects with a first degree relative with a past medical history of breast cancer will be excluded from the study.	To ensure the safety of enrolled subjects
Section 7.2.5 Table 5	Added amylase, lipase, apolipoprotein A, and apolipoprotein B to the chemistry panel and specified these will be blinded to the investigator.	For continued safety monitoring of enrolled subjects

Section #	Description of Change	Brief Rationale
Section 8.4.1	Specified that sensitivity and subgroup analyses of the primary efficacy endpoint will be provided in the SAP.	Clarification

14.5. Version 2.0: 14 March 2022

Overall Rationale for the Amendment

The primary purpose of this amendment is to change the formulation of study drug utilized in this study from capsules to oral solution.

Section #	Description of Change	Brief Rationale
Throughout	Minor editorial and document formatting revisions	Minor, therefore, have not been summarized.
Throughout	Assessment of urine biomarkers was added throughout the protocol.	Update as p75NTR fragment will be evaluated through urine, not CSF.
Section 1.2, Section 5.1, Section 5.2	Updates were done throughout to switch from the use of a capsule formulation of the study drug to an oral solution formulation.	To address the swallowing concerns in this subject population
Section 2.2.2, Section 2.2.4, Table 1, Table 2, Section 7, Section 7.1.1	Modified language as PFTs encompass the tests performed during this study, including SNIP. In addition, clarified that several tests will not be performed as part of PFTs.	Clarification
Section 3.2, Section 8.4, Section 8.4.1, Section 8.4.2, Section 8.4.3	Added language related to Part A and Part B being reported separately.	Clarification
Section 3.2, Table 1, Section 7.1.8.2	Removed language stating that subjects participating in the PK Sub-Study will sign an additional ICF.	The PK Sub-Study will not have a separate ICF.
Section 4.2	Revised criteria for ALS definition and minimum ALSFRS-R score for enrollment.	To broaden the enrollable patient population and ease site burden in identifying eligible patients
	Revised the time period prior to the start of Screening Period for concomitant medication use.	
Section 4.3	Revised the time period prior to the start of Screening Period for surgery.	To broaden the enrollable patient population and ease site burden in identifying eligible patients
	Clarified edaravone treatment, where applicable.	Clarification as edaravone is not approved for the treatment of ALS in some countries where this study is planned to be conducted
Table 1, Table 2	Day 14 and Day 183 visits were added for evaluation of clinical laboratory tests.	Visit added to incorporate FDA feedback to assess lipid profiles 14 days after initial dosing with PTC857.
	Several footnotes were added or modified for clarity.	Clarification
Section 6.4, Section 12.3	Modified language surrounding storage and destruction of biological samples.	Clarification

Section #	Description of Change	Brief Rationale
Section 7.2.5	Added language for which test results will be provided to the investigator.	Clarification
Section 8.5	Added section to clarify that no interim analysis is planned.	Clarification

15. REFERENCES

Barber, SC and Shaw, PJ. Oxidative Stress in ALS: Key Role in Motor Neuron Injury and Therapeutic Target. Free Radic Biol Med 2010;48(5):629-641.

Cedarbaum, JM and Stambler, N. Performance of the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) in Multicenter Clinical Trials. J Neurol Sci 1997;152 Suppl 1:S1-9.

Edaravone (MCl-186) ALS 19 Study Writing Group. 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.

FDA (2020). Code of Federal Regulations Title 21 Part 312: Investigational New Drug Application, Subpart D. Responsibilities of Sponsors and Investigators.

Ferrante, RJ, Browne, SE, Shinobu, LA, Bowling, AC, Baik, MJ, MacGarvey, U, et al. Evidence of Increased Oxidative Damage in Both Sporadic and Familial Amyotrophic Lateral Sclerosis. J Neurochem. 1997;69(5):2064-2074.

Jenkinson, C, Fitzpatrick, R, Brennan, C, Bromberg, M and 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;246 Suppl 3:Iii16-21.

Lin, MT and Beal, MF. Mitochondrial Dysfunction and Oxidative Stress in Neurodegenerative Diseases. Nature. 2006;443(7113):787-795.

Maarouf, CL, Beach, TG, Adler, CH, Shill, HA, Sabbagh, MN, Wu, T, et al. Cerebrospinal Fluid Biomarkers of Neuropathologically Diagnosed Parkinson's Disease Subjects. Neurol Res 2012;34(7):669-676.

Masaldan, S, Bush, AI, Devos, D, Rolland, AS and Moreau, C. Striking While the Iron Is Hot: Iron Metabolism and Ferroptosis in Neurodegeneration. Free Radic Biol Med 2019;133:221-233.

Oda, E, Ohashi, Y, Tashiro, K, Mizuno, Y, Kowa, H and Yanagisawa, N. Reliability and Factorial Structure of a Rating Scale for Amyotrophic Lateral Sclerosis. No To Shinkei [Brain and Nerve] 1996;48(11):999-1007.

Paganoni, S, Macklin, EA, Hendrix, S, Berry, JD, Elliott, MA, Maiser, S, et al. Trial of Sodium Phenylbutyrate-Taurursodiol for Amyotrophic Lateral Sclerosis. N Engl J Med 2020;383(10):919-930.

Petrov, D, Mansfield, C, Moussy, A and Hermine, O. ALS Clinical Trials Review: 20 Years of Failure. Are We Any Closer to Registering a New Treatment? Front Aging Neurosci 2017;9:68.

Reale, M, Pesce, M, Priyadarshini, M, Kamal, MA and Patruno, A. Mitochondria as an Easy Target to Oxidative Stress Events in Parkinson's Disease. CNS Neurol Disord Drug Targets 2012;11(4):430-438.

Riederer, P, Sofic, E, Rausch, WD, Schmidt, B, Reynolds, GP, Jellinger, K and Youdim, MB. Transition Metals, Ferritin, Glutathione, and Ascorbic Acid in Parkinsonian Brains. J Neurochem 1989;52(2):515-520.

Weiduschat, N, Mao, X, Hupf, J, Armstrong, N, Kang, G, Lange, DJ, et al. Motor cortex glutathione deficit in ALS measured in vivo with the J-editing technique. Neurosci Lett 2014;570:102-107.

Wenzel, SE, Tyurina, YY, Zhao, J, St Croix, CM, Dar, HH, Mao, G, et al. PEBP1 Wardens Ferroptosis by Enabling Lipoxygenase Generation of Lipid Death Signals. Cell 2017;171(3):628-641 e626.

Ye, LF and Stockwell, BR. Transforming Lipoxygenases: PE-Specific Enzymes in Disguise. Cell 2017;171(3):501-502.

16. APPENDICES

16.1. Prohibited Medications During the Course of the Study

The results of an in vitro study indicated that multiple CYP enzymes (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) may be involved in PTC857 metabolism. Since multiple CYPs are involved in the metabolism of PTC857, inhibition/induction of any single enzyme may not have significant effect.

Hence, the strong and moderate inhibitors/inducers of the putative CYP enzymes that metabolize PTC857 listed in Table 10 and Table 11 may be used per the treating physician's discretion (ie, are not prohibited). If the use of any of these medicines is necessary, greater attention should be exercised for monitoring subject safety. If any SAEs are observed, an 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.

The results of a clinical drug-drug interaction study evaluating the effect of PTC857 on CYP1A2, CYP2B6, and CYP3A4 substrates (PTC857-DDI-101-HV) indicated mild to moderate changes in exposure of the respective substrates, hence, no dose adjustments are necessary when drugs metabolized by these enzymes are co-administered with PTC857.

The combination of edaravone plus sodium phenylbutyrate/taurursodiol is not permitted for enrollment to this study. Furthermore, the use of any non-approved form of sodium phenylbutyrate and/or taurursodiol for the treatment of ALS is also excluded from this study.

Subjects may not begin standard-of-care therapy (riluzole, edaravone, or sodium phenylbutyrate/taurursodiol) for ALS during the placebo-controlled Treatment Period (Part A).

Upon entry to the LTE Period, participants may continue their established standard-of-care treatment and/or initiate treatment with standard-of-care therapy based on their treating physician's discretion where applicable or available without restriction to the number or combination of therapies.

Table 10: Strong/Moderate Inhibitors of CYP Enzymes

CYP Enzymes	Strong Inhibitors	Moderate Inhibitors
CYP1A2	Ciprofloxacin, enoxacin, fluvoxamine	-
CYP2B6	-	-
CYP2C8	Gemfibrozil	Clopidogrel, deferasirox, teriflunomide
CYP2C9	-	Amiodarone, fluconazole, miconazole, piperine
CYP2C19	Fluconazole, fluoxetine, fluvoxamine, ticlopidine	Felbamate
CYP2D6	Bupropion, fluoxetine, paroxetine, quinidine, terbinafine	Abiraterone, cinacalcet, duloxetine, lorcaserin, mirabegron
CYP3A4	Boceprevir, cobicistat, danoprevir and ritonavir, elvitegravir and ritonavir, grapefruit juice, indinavir and ritonavir, itraconazole, ketoconazole, lopinavir and ritonavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, telithromycin, troleandomycin, voriconazole, clarithromycin, idelalisib, nefazodone, nelfinavir	-

Abbreviations: AUC, area under the concentration-time curve; CYP, cytochrome P450; DDI, drug-drug interaction
Note: Inhibitors predictably inhibit metabolism via a given pathway and are commonly used in prospective clinical DDI studies. Strong and moderate inhibitors are drugs that increase the AUC of sensitive substrates of a given metabolic pathway ≥5-fold and ≥2 to <5-fold, respectively.

Table 11: Strong/Moderate Inducers of CYP Enzymes

CYP Enzymes	Strong Inducers	Moderate Inducers
CYP1A2	-	-
CYP2B6	Carbamazepine	-
CYP2C8	-	Rifampin
CYP2C9	-	Enzalutamide, rifampin
CYP2C19	Rifampin	Apalutamide, efavirenz enzalutamide, phenytoin
CYP3A4	Apalutamide, carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, St. John's wort	-

Abbreviations: AUC, area under the concentration-time curve; CYP, cytochrome P450; DDI, drug-drug interaction
Note: Inducers predictably induce metabolism via a given pathway and are commonly used in prospective clinical DDI studies. Strong and moderate index inducers are drugs that decreases the AUC of sensitive substrates of a given metabolic pathway by ≥80% and ≥50% to <80%, respectively.