STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER:

CY 4033

STUDY TITLE:

A Phase 3, Open-Label Extension Study of Tirasemtiv for Patients with Amyotrophic Lateral Sclerosis (ALS) who Completed VITALITY-ALS (CY 4031)

STUDY DRUG:

Tirasemtiv

VERSION: 1.0

DATE OF PLAN:

24 October 2017

BASED ON:

Protocol Dated on 13 July 2016

SPONSOR:

Cytokinetics, Inc.

280 East Grand Avenue,

South San Francisco, CA 94080

Tel No. 650 624 3000

This study is being conducted in compliance with good clinical practice, including the archiving of essential documents.

SIGNATURE PAGE

* This document has been reviewed and approved by:

Long Ma, Ph.D.

Senior Biostatistician, Biometrics
Cytokinetics, Inc.

Lisa Meng, Ph.D.

Senior Director, Biometrics
Cytokinetics, Inc.

Bettina Cockroft, M.D., Ph.D.

Vice President, Clinical Research
Cytokinetics, Inc.

*See electronic signatures at the end of the document.

TECHNICAL SUMMARY REPORT (TSR)

Name of Sponsor/Company **Cytokinetics, Inc.**	**Individual Study Table Referring to Part of the Dossier:** **Volume:**	***(For National Authority Use Only)*:**
Name of Finished Product: Tirasemtiv	**Page:**	
Name of Active Ingredient: CK-2017357		
Title of Study: A Phase 3, Open-Label Extension Study of Tirasemtiv for Patients with Amyotrophic Lateral Sclerosis (ALS) who Completed VITALITY-ALS (CY 4031)		
Investigators: Study Center(s):		
Studied Period (years): up to 6 years	**Phase of Development:** Phase 3 - Open Label Extension	

Objectives:

Primary:

The primary objective is to assess the long-term safety and tolerability of tirasemtiv, in patients with Amyotrophic Lateral Sclerosis (ALS).

Secondary:

1. To compare the clinical course of patients who completed treatment with tirasemtiv in CY 4031 with those who completed treatment with placebo in CY 4031 during continued treatment of both groups with tirasemtiv during CY 4033.
2. To compare the clinical course of patients who completed treatment with tirasemtiv in CY 4031 during that study with their clinical course during continued treatment with tirasemtiv during CY 4033.
3. To compare the clinical course of patients who completed treatment with placebo in CY 4031 during that study with their clinical course during treatment with tirasemtiv during CY 4033.

Methodology:

CY 4033 is an open-label extension study of tirasemtiv in patients with ALS who completed participation (through Week 56) in CY 4031 (VITALITY-ALS). Following enrollment, patients will begin dosing with tirasemtiv 125 mg twice daily (250 mg/day) for 4 weeks and will titrate to their maximum tolerated dose with an increase to 375 mg/day at Week 4 and (if 375 mg/day is tolerated) to 500 mg/day at Week 6, the maximum dose being 250 mg twice daily (500 mg/day). Patients can down-titrate to a tolerated dose of tirasemtiv at any time. Clinic visits will occur at Day 1, Week 4, Week 8, Week 12, and every 12 weeks thereafter. Slow vital capacity (SVC), the ALS Functional Rating Scale (as revised; i.e., the ALSFRS-R), laboratory evaluations, vital signs, adverse events, and other related assessments will be conducted at each clinic visit.

Number of Patients (Planned and Analyzed):

Approximately 350 patients who completed CY 4031 through the Follow-up Visit are expected to be enrolled in this open-label extension study.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Inclusion criteria:

1. Able to comprehend and willing to sign an Informed Consent Form.
2. Completed participation on study drug and the Follow-up Visit in CY 4031 study

Exclusion criteria:

1. Has a diaphragm pacing system (DPS) at study entry or anticipate DPS placement during the course of the study.
2. Has taken any investigational study drug (other than tirasemtiv) prior to dosing, within 30 days or five half-lives of the prior agent, whichever is greater.
3. Use of tizanidine and theophylline-containing medications during study participation.
4. Participation or planning to participate in another clinical trial involving stem cell therapy for the treatment of ALS or another investigational drug

Test Product, Dose and Mode of Administration:

CK-2017357 (tirasemtiv) investigational product is supplied as immediate release, white, modified oval tablets containing 125 mg of tirasemtiv which are to be stored under secure conditions at 15-30°C; 59-86°F. Tirasemtiv tablets will be supplied to the clinical site in induction sealed 60 cc white high-density polyethylene bottles containing 60 tablets per bottle. Bottles will be labeled with a lot number.

In this open label extension study with tirasemtiv, the maximum dose level for all patients will be 500 mg/day. Initiation and titration of dosing will use the following schedule:

- Treatment for 4 weeks at 250 mg/day (125 mg twice daily)
- If tolerated, up-titration to 375 mg/day (125 mg in the morning and 250 mg in the evening) for 2 weeks
- If tolerated, up-titration to the final dose of 500 mg/day (250 mg twice daily)

Duration of Treatment:

Clinic visits will occur at Day 1, Weeks 4, 8, and 12, and every 12 weeks thereafter.

Reference Therapy, Dose and Mode of Administration:

Not applicable.

Criteria for Evaluation:

Safety Endpoints:

The long-term safety and tolerability of tirasemtiv will be assessed by the incidence of adverse events (AEs). Additional safety evaluations will include clinical laboratory results, vital signs, and suicidality assessments.

Efficacy Endpoints:

- Time to first use of assisted ventilation or death
- Time to the first occurrence of respiratory insufficiency (defined as tracheostomy or the use of non-invasive ventilation for ≥ 22 hours per day for ≥ 10 consecutive days) or death
- Time to death
- Change in percent predicted SVC from baseline
- Change in ALSFRS-R score from baseline
- Slope of changes from baseline in percent predicted SVC
- Slope of changes from baseline in ALSFRS-R

The time-to-event endpoints will be assessed from the baseline of CY 4031 and from the baseline of CY 4033 to the end of CY 4033. The change-from-baseline endpoints will be assessed from baseline of CY 4031 and from baseline of CY 4033 to Week 24 and Week 48 of CY 4033. The slope of change from baseline endpoints will be assessed during the first 24 weeks and first 48 weeks of either CY 4031 or CY 4033.

Statistical Methods:

Patients who received tirasemtiv at the first randomization in CY 4031 and who therefore continue to receive tirasemtiv during CY 4033 will be considered the "early start" treatment group. Patients who received placebo at the first randomization in CY 4031 and who therefore initiate tirasemtiv during CY 4033 will be considered the "delayed start" treatment group. Summary statistics (by treatment group and overall) for continuous endpoints will include numbers of patients, means, medians, standard deviations, minima, and maxima. For categorical endpoints, frequencies and percentages will be given. For time-to-event endpoints, the number of patients at risk, number of events, the median and Greenwood's 95% confidence intervals (CI) of the median, and quartiles will be provided by Kaplan-Meier method.

The number and percentage of patients reporting treatment-emergent adverse events (TEAEs) will be summarized by system organ class and preferred term for the early and delayed start treatment groups and overall. In addition, the number and percentage of patients reporting TEAEs will be tabulated by system organ class, preferred term, and severity for the early and delayed start treatment groups and overall.

The change from baseline at each clinic visit will be analyzed using Mixed-Effect Model Repeated Measures (MMRM) model-based method which will include treatment group, baseline value, visit, pooled site, and riluzole use/non-use during the double-blind, placebo-controlled phase in CY 4031 as well as interaction terms for treatment group-by-baseline and treatment group-by-visit for efficacy endpoints. The least-squares mean of the difference between the treatment groups, standard error of the difference, and 95% CI of the difference will be presented.

Slope endpoints will be analyzed using a mixed model which will include treatment group, baseline value, pooled site, time from baseline, riluzole use/non-use in the double-blind, placebo-controlled phase in CY 4031 as well as interaction terms for treatment group-by-baseline, and treatment group-by-time, assuming random slope effect. The estimated slope and the slope difference as well as their standard errors will be presented.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
ALS	amyotrophic lateral sclerosis
ALSFRS-R	ALS Functional Rating Scale-Revised
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATC	anatomical therapeutic chemical
BDI	Beck depression inventory
BLQ	below the lower limit of quantitation
CI	confidence interval
eCRF	electronic case report form
CSR	clinical study report
EAS	efficacy analysis set
EDC	electronic data capture
FAS	full analysis set
ID	identification
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities Terminology
MMRM	mixed-effect model repeated measures
NIV	non-invasive ventilation
NCI-CTCAE	the National Cancer Institute Common Terminology Criteria for Adverse Events
PCS	potential clinical significance
PD	pharmacodynamic
PK	pharmacokinetics
PT	preferred term
Q1	first quartile
Q3	third quartile
SD	standard deviation
SAE	serious adverse event
SAS	safety analysis set
SAP	statistical analysis plan

Abbreviation	Term
SOC	system organ class
SVC	slow vital capacity
TEAEs	treatment-emergent adverse events
TFL	tables, figures and listings
WHO	World Health Organization

1. INTRODUCTION

This statistical analysis plan (SAP) describes the statistical analysis methods and data presentations to be used in tables, figures and listings (TFL) in the clinical study report (CSR) for CY 4033 study. This SAP is based on the study protocol dated on 13 July, 2016. The SAP will be finalized before database finalization for CY 4031. Any changes made after the finalization of the SAP will be documented in an amended version of the SAP and will be tracked in the revision history.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study Objectives

2.1.1. Primary Objective

The primary objective is to assess the long-term safety and tolerability of tirasemtiv, in patients with Amyotrophic Lateral Sclerosis (ALS).

2.1.2. Secondary Objectives

The secondary objectives are:

- To compare the clinical course of patients who completed treatment with tirasemtiv in CY 4031 with those who completed treatment with placebo in CY 4031 during continued treatment of both groups with tirasemtiv during CY 4033
- To compare the clinical course of patients who completed treatment with tirasemtiv in CY 4031 during that study with their clinical course during continued treatment with tirasemtiv during CY 4033
- To compare the clinical course of patients who completed treatment with placebo in CY 4031 during that study with their clinical course during treatment with tirasemtiv during CY 4033

2.2. Study Endpoints

2.2.1. Safety Endpoints

The long-term safety and tolerability of tirasemtiv will be assessed primarily through the incidence of adverse events (AEs) during the study. Additional safety evaluations will include clinical laboratory results, vital signs, and suicidality assessments.

2.2.2. Efficacy Endpoints

There will be two analysis sets for the efficacy endpoints:

1. The analyses for the efficacy endpoints with the reference baseline in CY 4031 will be based on the population of all patients in the Full Analysis Set (FAS) in CY 4031.
2. The analyses for the efficacy endpoints with the reference baseline in CY 4033 will be based on the Efficacy Analysis Set (EAS) defined in Section 5.4.4. The analyses for selected efficacy endpoints from baseline in CY 4031 based on EAS will be conducted as well.

2.2.2.1. Time-to-Event Endpoints

There will be two analysis timeframes for time-to-event endpoints: 1) From baseline in CY 4031 to the end of CY 4033; 2) From baseline in CY 4033 to the end of CY 4033.

Time-to-event endpoints include the following:

1. Time to the first use of assisted ventilation or death. Assisted ventilation is defined as invasive (tracheostomy) or non-invasive ventilation (NIV) for at least 2 hours over a 24-hour period for at least 5 consecutive days.
2. Time to the first occurrence of respiratory insufficiency (defined as tracheostomy or the use of non-invasive ventilation for ≥ 22 hours per day for ≥ 10 consecutive days) or death
3. Time to death

2.2.2.2. Change-from-Baseline Endpoints

Change-from-baseline endpoints will be presented descriptively only if one third of patients remain in either of the delayed start or early start treatment groups in CY 4033 at the time points indicated below.

Change-from-baseline endpoints:

1. Change in percent predicted slow vital capacity (SVC) (Knudson, Lebowitz et al. 1983) from baseline in CY 4031 to Week 24 and to Week 48 of CY 4033
2. Change in percent predicted SVC from baseline in CY 4033 by visit in CY 4033
3. Change in ALS Functional Rating Scale-Revised (ALSFRS-R) (Cedarbaum, Stambler et al. 1999) total score from baseline in CY 4031 to Week 24 and to Week 48 of CY 4033
4. Change in ALSFRS-R total score from baseline in CY 4033 by visit in CY 4033

2.2.2.3. Slope Endpoints

Slopes of changes from baseline in percent predicted SVC and in ALSFRS-R total score during the first 24 and first 48 weeks of CY 4033 include the following:

1. Slopes of changes from CY 4031 baseline in percent predicted SVC to Week 24 and to Week 48 in CY 4033
2. Slopes of changes from CY 4033 baseline in percent predicted SVC to Week 24 and to Week 48 in CY 4033
3. Slopes of changes from CY 4031 baseline in ALSFRS-R total score to Week 24 and to Week 48 in CY 4033
4. Slopes of changes from CY 4033 baseline in ALSFRS-R total score to Week 24 and to Week 48 in CY 4033

2.2.3. Additional Time-to-Event Endpoints

The following time-to-event endpoints will be analyzed using the two timeframes defined in Section 2.2.2.1:

1. Time to the first occurrence of a decline from baseline in percent predicted SVC ≥ 20 percentage points or the onset of respiratory insufficiency (defined as tracheostomy or the first day of the use of non-invasive ventilation for ≥ 22 hours per day for ≥ 10 consecutive days) or death

2. Time to the first occurrence of a decline from baseline in percent predicted SVC ≥ 20 percentage points or the first use of mechanical ventilatory assistance or death. Mechanical ventilatory assistance is defined as invasive or non-invasive ventilation for at least 2 hours over a 24-hour period for at least 5 consecutive days
3. Time to the first occurrence of a decline from baseline in SVC to ≤ 50 percent predicted or the onset of respiratory insufficiency (defined as tracheostomy or the first day of the use of non-invasive ventilation for ≥ 22 hours per day for ≥ 10 consecutive days) or death
4. Time to the first occurrence of a decline from baseline in SVC to ≤ 50 percent predicted or first use of mechanical ventilatory assistance or death.
5. Time to the initiation of regular use of non-invasive ventilation or death. The regular use of non-invasive ventilation is defined as non-invasive ventilation use for at least 2 hours over a 24-hour period for at least 5 consecutive days.
6. Time to the first occurrence of a two-point or greater drop in the composite of the respiratory components of the ALSFRS-R (i.e., items 10, 11, and 12) from baseline or death.

2.2.4. Additional Change-from-Baseline and Slope Endpoints

1. Change from CY 4031 baseline in the respiratory sub-score of the ALSFRS-R (i.e., the sum of scores for items 10, 11, and 12) to Week 24 and to Week 48 in CY 4033
2. Change from CY 4033 baseline in the respiratory sub-score of the ALSFRS-R (i.e., the sum of scores for items 10, 11, and 12) by visit in CY 4033
3. Slopes of changes from CY 4031 baseline in the respiratory sub-score of the ALSFRS-R (i.e., the sum of scores for items 10, 11, and 12) to Week 24 and to Week 48 in CY 4033
4. Slopes of changes from CY 4033 baseline in the respiratory sub-score of the ALSFRS-R (i.e., the sum of scores for items 10, 11, and 12) to Week 24 and to Week 48 in CY 4033

3. STUDY DESIGN

3.1. Summary of Study Design

CY 4033 is a multi-national, open-label extension treatment study with the selective fast skeletal muscle troponin activator, tirasemtiv, in patients with ALS who completed participation (through Week 56) in the VITALITY-ALS (CY 4031) study. Following enrollment, patients will begin dosing with tirasemtiv 125 mg twice daily (250 mg/day) for 4 weeks and will titrate to their maximum tolerated dose with an increase to 375 mg/day at Week 4 and (if 375 mg/day is tolerated) to 500 mg/day at Week 6, the maximum dose being 250 mg twice daily (500 mg/day). Patients who do not tolerate a higher dose after up-titration may return to a previously tolerated dose at any time. No upward dose adjustments are allowed after a dose reduction.

Table 1: Study Dosing Plan

Initial Dose (Day 1 Clinic Visit)	Planned Up-Titration (Week 4 Clinic Visit)	Planned Up-Titration (Week 6 Phone Call)
250 mg tirasemtiv total daily dose First dose: 1 tablet (125 mg) of tirasemtiv Second dose: 1 tablet (125 mg) of tirasemtiv (approximately 12 hours apart)	*375 mg tirasemtiv total daily dose* First dose: 1 tablet (125 mg) of tirasemtiv Second dose: 2 tablets (250 mg) of tirasemtiv (approximately 12 hours apart)	*500 mg tirasemtiv total daily dose* First dose: 2 tablets (250 mg) of tirasemtiv Second dose: 2 tablets (250 mg) of tirasemtiv (approximately 12 hours apart)

Clinic visits will occur at Day 1, Week 4, Week 8, Week 12, Week 24, Week 36, Week 48, and every 12 weeks thereafter. Patients will be contacted by phone to assess their tolerance of tirasemtiv at Week 2, Week 6, and Week 10. If a patient discontinues tirasemtiv, the patient will come to the clinic for the Tirasemtiv Discontinuation Visit and the 28 Day Safety Follow-up Visit if the patient is able; otherwise the patient will be contacted by phone for these visits. Slow vital capacity (SVC), the ALS Functional Rating Scale (as revised; i.e., the ALSFRS-R), laboratory evaluations, vital signs, adverse events, and other related assessments will be conducted at each clinic visit.

3.2. Definition of Study Drugs

The investigational product or study drug in CY 4033 is CK-2017357 (tirasemtiv).

3.3. Sample Size Considerations

Approximately 350 patients who completed CY 4031 through the Follow-up Visit are expected to be enrolled in this open-label extension study, CY 4033.

3.4. Clinical Assessments

Clinical safety assessments, including clinical laboratory evaluations, vital signs and suicidality assessment (Beck Depression Inventory-Fast Screen) (Beck, Steer et al. 1996), and efficacy assessments, including ALSFRS-R and SVC, are listed in Schedule of Events (Appendix 1).

4. PLANNED ANALYSES

4.1. Interim Analyses

CY 4033 is an open-label extension study of tirasemtiv in patients with ALS who completed CY 4031. After database lock and unblinding of study CY 4031, an interim analysis of CY 4033 will be performed.

Additional interim analyses also may be conducted after all patients reach study time point milestones. Possible milestones include (but are not limited to): Week 24, Week 48, and Week 96. Trigger criterion for these potential milestones will be based upon emerging safety and tolerability data from the CY 4033 study. A description of any interim analyses conducted while the study is ongoing will be specified in separate statistical analysis plan(s). Each interim analysis plan will provide the details of data cut-off, including data cutoff date, data extraction date, data cleaning and extraction specifications.

4.2. Final Analysis

The final analysis of the data will be performed after all patients discontinue study participation and final database lock.

5. GENERAL CONSIDERATIONS FOR DATA ANALYSES AND HANDLING

5.1. General Summary Table and Individual Patient Data Listing Considerations

Analysis results will be presented using descriptive statistics by early start (the group of patients who received tirasemtiv at the first randomization in CY 4031) and delayed start (the group of patients who received placebo at the first randomization in CY 4031 and began tirasemtiv during CY 4033) treatment groups and overall in summary tables in the safety and efficacy analysis sets. For continuous variables, the number of patients, mean, standard deviation (SD), median, minimum and maximum will be presented; for categorical variables, the number and percentage of patients in each category will be presented; time-to-event endpoints will be summarized using Kaplan-Meier method (Kaplan and Meier 1958) (first quartile (Q1), third quartile (Q3), median, and corresponding Greenwood's 95% confidence intervals (CIs) (Kalbfleisch and Prentice 2011). For model-based analysis, least-squares mean, difference of least-squares means between the early start and delayed start treatment groups, their standard errors and 95% CIs, and 2-sided p-values for the relative statistical inferences will be presented. The above comparisons will be performed by the maintenance dose level defined in Section 8.3.3.

All statistical tests will be 2-sided and performed at the 5% significance level unless otherwise specified.

Data will be presented in by-patient listings for all patients unless otherwise specified. All by-patient listings will be sorted by treatment group, patient ID and visit and will include demographics (age at baseline of CY 4033 and sex), maintenance dose level, in ascending order.

5.2. Data Management

Data will be entered into an electronic data capture (EDC) system with programmed edit checks to insure integrity. Any missing data and potential data issues will be queried to the participating clinical sites. Laboratory tests will be conducted by a central laboratory with results entered into database managed by the central laboratory.

5.3. Data Presentation Conventions

The following conventions are applied to all data presentations and summaries.

- For continuous variables, all mean and median values are formatted to one more decimal place than the measured value. Standard deviation values are formatted to two more decimal places than the measured value. Minimum and maximum values are presented with the same number of decimal places as the measured value.
- For categorical variables, the number and percentage of responses are presented in the form XX (XX.X%).
- P-values, if applicable, will be presented to 4 decimal places. If the p-value is less than 0.0001 then it will be presented as < 0.0001. If the rounded result is a value of 1.000, it will be displayed as > 0.9999.

5.3.1. Data Handling for Pharmacokinetics Data

Natural logarithm transformation will be used for the analysis of plasma/blood concentrations. Plasma concentration values that are below the lower limit of quantitation (BLQ) will be presented as "BLQ" in the concentration data listing.

5.4. Analysis Populations

Analysis populations define the patients to be included in an analysis. Analysis sets and their definitions are provided in this section. For each analysis set, the number and percentage of patients eligible for inclusion, as well as the number and percentage of patients who were excluded and the reasons for their exclusion, will be provided by early start and delayed start treatment groups.

5.4.1. Screen Failures

Not applicable. Patients who signed the Informed Consent Form but did not receive the study medication will be listed and excluded from analysis.

5.4.2. Safety Analysis Set

The Safety Analysis Set (SAS) will consist of all patients who were enrolled and received at least one dose of tirasemtiv in CY 4033.

5.4.3. Full Analysis Set

The Full Analysis Set will consist of all randomized patients who received at least one dose of study medication during the randomized double-blind, placebo-controlled phase and had at least one post-randomization efficacy assessment in CY 4031, regardless of whether or not they continued into CY 4033.

5.4.4. Efficacy Analysis Set

The Efficacy Analysis Set will consist of all patients who completed CY 4031, were enrolled in CY 4033, received at least 1 dose of tirasemtiv, and had at least one post-baseline efficacy outcome assessment during CY 4033. If there is a gap of more than 60 days between the Week 56 Follow-up Visit in CY 4031 and Day 1 in CY 4033, and the patient does not have a Day 1 assessment in CY 4033, then the patient will be excluded in the efficacy analysis set.

5.4.5. Pharmacokinetic Analysis Set

The Pharmacokinetic (PK) Analysis Set includes all patients who were received at least 1 dose of tirasemtiv and have at least 1 measurable post-dose concentration value for tirasemtiv, provided they have no major protocol violations that could affect the PK of tirasemtiv in CY 4033.

5.4.6. Pharmacodynamic Analysis Set

The Pharmacodynamic (PD) Analysis Set includes all patients who received at least 1 dose of tirasemtiv and have at least 1 measurable post-dose tirasemtiv plasma concentration and at least one measurable post-baseline assessment of at least one the PD endpoints in CY 4033.

5.5. Baseline Definition

5.5.1. Baseline for Efficacy Endpoints

Two baseline values will be used for change-from-baseline endpoints:

- The CY 4033 baseline value refers to the assessment at the Day 1 in CY 4033. If the assessment at the Day 1 in CY 4033 is not available, and no more than 60 days have elapsed since the assessment at the Follow-up Visit (Week 56) in CY 4031, the assessment at the Follow-up Visit (Week 56) in CY 4031 will be the baseline in CY 4033.
- The CY 4031 baseline refers to the last available assessment prior to the first dose of the open-label lead-in phase in CY 4031.

Two reference dates will be used for time-to-event endpoints:

- The date of first dose of study drug in the double-blind, placebo-controlled phase of CY 4031
- The date of first dose of study drug in CY 4033

5.5.2. Baseline for Safety Endpoints

Baseline for clinical laboratory assessments, suicidality assessment and vital signs are defined as the last non-missing assessment prior to the first dose of study drug in CY 4033.

5.6. Derived and Transformed Data

5.6.1. Time Point and Durations

1. Two timeframes for time-to-event endpoints will be used in this study:

   Time-to-event days relative to reference date = event date – reference date + 1

   The reference date is the date of first dose of study drug in double-blind, placebo-controlled phase in CY 4031 or the date of first dose of tirasemtiv in CY 4033.

2. Days to AE onset:

   Days to AE onset = onset date – first dose date in CY 4033 + 1

   Days to AE onset = onset date – first dose date in CY 4033 (if onset is before the date of first dose of CY 4033)

3. Days of change-from-baseline

   Days from CY 4031 baseline = post-baseline assessment date – assessment date at CY 4031 baseline + 1

   Days from CY 4033 baseline = post-baseline assessment date – assessment date at CY 4033 baseline + 1

5.6.2. Time-to-Events Endpoints

Patients will be censored at the end of study if the patient does not experience an event prior to date of the last study contact.

5.6.3. ALS Functional Rating Scale-Revised

The ALS Functional Rating Scale-Revised (ALSFRS-R) assessments will be conducted on Screening, Open-label Lead-in phase, Double-Blind, Placebo Controlled Phase, Double-Blind, Placebo-Controlled, tirasemtiv Withdrawal Phase and Week 56 Follow up in CY 4031, and on Day 1, Week 4, Week 8, Week 12 and every 12 weeks thereafter and at the 28-day Follow-up Clinical Visit in CY 4033. The ALSFRS-R total score is the sum of the scores of the 12 individual questions from all domains. Subtotal scores will also be calculated for each domain:

- Bulbar domain: (1) speech, (2) salivation, (3) swallowing
- Fine motor domain: (4) handwriting, (5) cutting food and handling utensils, (6) dressing hygiene
- Gross motor domain: (7) turning in bed and adjusting bedclothes, (8) walking, (9) climbing stairs
- Respiratory domain: (10) dyspnea, (11) orthopnea, (12) respiratory insufficiency

The total score of ALSFRS-R cannot be calculated if the answer to any of the 12 individual questions is missing. Subtotal score will be assigned to missing if any question in the domain is missing.

5.6.4. Respiratory Function

Respiratory function will be analyzed by percent predicted SVC. The respiratory function assessments are conducted on Screening, Open-label Lead-in phase, Double-Blind, Placebo Controlled Phase, Double-Blind, Placebo-Controlled, tirasemtiv Withdrawal Phase and Week 56 Follow up in CY 4031, and on Day 1, Week 4, Week 8, Week 12 and every 12 weeks thereafter at the 28-day Follow-up Clinical Visit in CY 4033. SVC is collected at each clinical visit. The highest result from trials 1, 2, and 3 for SVC, measured in liters at each clinical visit as recorded on the eCRFs, and the calculated percent predicted SVC will be used for analysis.

5.6.5. Change from Baseline

Baseline for efficacy endpoints are defined in the Section 5.5.

Change from baseline = post-baseline assessment value – baseline value

5.6.6. Analysis Visit Windows

Nominal visits in CY 4033 will be used for all analyses. Nominal visits in CY 4031 will be used in the comparisons of clinical course of patients in CY 4031 and CY 4033. Please see the section of Analysis Visit Windows for the definition of nominal visits in the SAP for CY 4031. Unscheduled visits in CY 4033 will be assigned to the closest nominal visit if data at the nominal visit is not available. The lower limit and upper limit of each nominal visit is listed in Table 2 below.

Table 2: Analysis Visit Windows in CY 4033 (Days)

Nominal Visit	Nominal Day	Lower Limit	Upper Limit
Day 1	1	1	1
Week 4	29	2	42
Week 8	57	43	70
Week 12	85	71	126
Week 12*n	12*n*7+1	12*(n-0.5)*7+1	12*(n+0.5)*7

- Day displayed for these nominal visits is calculated relative to Day 1 dosing in CY 4033
- n: start from 2

5.6.7. Multiple Assessments within Visit Windows

If multiple non-missing numeric observations exist in an analysis window or with same nominal visit name, records closest to the nominal day will be chosen if a single value is needed. The later measurement will be used if there are ties.

5.7. Handling of Missing Data

5.7.1. Missing Efficacy Endpoints

The handling of missing efficacy endpoints is described in Section 7.5.3.

5.7.2. Missing Start and Stop Dates for Prior and Concomitant Medication

No imputation of missing/partial dates will be performed. The available year or year and month in a partial date will be used and will be compared to first dosing year, month and day to determine whether to include the medication in the medication history or as a concomitant medication. If the available data do not give sufficient information to classify the medication, the medication will be classified as a concomitant medication.

5.7.3. Missing Start and Stop Dates for Adverse Events

For AEs with incomplete date information recorded in the eCRF, date/time imputation will follow the algorithm below:

1. For missing AE onset dates and times:
    - If the AE onset date is missing and the month of AE onset is after the month of the first dosing date, then the first day of the month of AE onset will be the imputed date of AE onset.
    - If the AE onset date is missing and the month of AE onset is the same as the month of the first dosing date in CY 4033 and the AE onset is after the first dosing date, then the first dosing date in CY 4033 will be the imputed AE onset date.
    - If no AE onset information is available and the AE was not reported in CY 4031, then the first dosing date in CY 4033 will be the imputed AE onset date.

- If the AE which was carryover from CY 4031 is without AE onset information, then the AE onset date will follow the imputation rules defined in Section 8.2 of CY 4031 SAP.

2. For missing AE end dates and times:
   - If the AE end date is missing and the AE end month is earlier than that of the Follow-up Visit, then the last day of the AE end month will be the imputed as the AE end date.
   - If the AE end date is missing and the AE end month is the same as that of the Follow-up Visit, then the date of the Follow-up Visit will be the imputed as the AE end date.
   - If no AE end information is available, then AE end date will not be imputed.

6. STUDY POPULATION

6.1. Patients Disposition

Patient disposition will be presented for the SAS. The number and percentage of patients who completed CY 4031, and who did not receive any study drug, who received at least one dose of study drug, who discontinued from treatment, and who discontinued from the study will be presented by early start and delayed start treatment group and overall in CY 4033.

Reasons for dose discontinuation as recorded on the End of Dosing page, and reasons for the study discontinuation as recorded on the End of Study page of the eCRF will be summarized as well.

A listing of patient disposition will be provided for the SAS. The listing will include the following: date of Week 56 Follow-up in CY 4031, dates of first and last dose in CY 4033, dates of final follow-up visit/last study contact, date of death, duration of tirasemtiv in CY 4031 and CY 4033 and in CY 4033 alone, demographic information (including age on CY 4033 Day 1), dose received at the time of tirasemtiv discontinuation, and reason for study discontinuation in CY 4033. The listing will be sorted by patient ID number in ascending order.

6.2. Protocol Deviations

A protocol deviation is any divergence from the protocol that impacts a patient's safety, or welfare or materially reduces the quality or completeness of the data.

The number and percentage of patients meeting any of the following protocol deviation criteria will be summarized by type of deviation, by the early start and delayed start treatment groups, and overall:

1. Patient was enrolled even though they did not satisfy inclusion and exclusion criteria;
2. Patient developed withdrawal criteria during the study but was not withdrawn;
3. Patient received the incorrect dose of tirasemtiv*;*
4. Patient took more than 50 mg riluzole while taking tirasemtiv*;*
5. Patient received an excluded concomitant treatment.

A listing will be provided by patient ID in ascending order.

6.3. Demographic and Baseline Characteristics

6.3.1. Demographics and Baseline Characteristics

Demographic and baseline characteristics will be summarized by the early start and delayed start treatment groups and overall as well as by the maintenance dose level for the SAS. Summaries will also will be provided for the EAS if the number of patients in the SAS is 10% more than the number of patients in the EAS. Patient demographic and baseline characteristics will include the following: age at Day 1 in CY 4033, gender, race, height (in cm), body weight in (kg) and body mass index (BMI, in kg/m^2).

A listing will be provided by patient ID in ascending order.

6.3.2. Other Baseline Characteristics

Other baseline characteristics include ALSFRS-R total and individual domain scores, and percent predicted SVC baselines in CY 4031 and in CY 4033. These baseline characteristics will be summarized by early start and delayed start treatment groups and overall using descriptive statistics (n, mean, SD, median, minimum, and maximum) for continuous variables and using the frequency counts and percentages of patients for categorical variables for the SAS. Summaries will also be provided for the EAS if the number of patients in the SAS is 10% more than the number of patients in the EAS.

6.4. Listing of Patient Inclusion and Exclusion Criteria

A listing of patients who do not meet inclusion and/or exclusion criteria will be provided by patient ID in ascending order.

6.5. Medical Conditions Present at Entry

Adverse events that are ongoing at the time of enrollment in Study CY 4033 are being collected using a Prior Adverse Events eCRF page and will be summarized as Adverse Events in CY 4033.

6.6. Prior Medication History and Medications Present at Entry

Medications started during the CY 4031 study and without stopping dates or after the first dose of study drug in CY 4033, or started during CY 4033 will be summarized as Concomitant Medications in CY 4033 (Section 8.4).

6.7. Baseline Physical Examination

Physical examination will be conducted at Day 1 visit in CY 4033 only.

6.8. Baseline Vital Signs

Baseline vital signs in CY 4033 will be the assessments at Day 1 in CY 4033. Baseline vital signs will be summarized and provided in the tables and listings along with post-baseline vital signs in Section 8.6.

6.9. Baseline Laboratory Data

Baseline laboratory data including hematology, serum chemistry, and urinalysis in CY 4033 will be the assessments at Day 1 in CY 4033. The baseline laboratory data in CY 4033 will be summarized and provided in the tables and listings along with post-baseline laboratory data Section 8.5.

6.10. Baseline Efficacy Evaluations

The baseline efficacy endpoints including percent predicted SVC and ALSFRS-R baseline values in CY 4031 and in CY 4033 defined in Section 5.5.1 will be summarized and presented in the tables and listings along with post-baseline efficacy assessments.

7. EFFICACY

7.1. General Considerations

Efficacy analysis will be based on the EAS unless otherwise specified.

Two efficacy baseline values for change-from-baseline endpoints defined in Section 5.5.1 will be used in this study. Two timeframes defined in Section 2.2.2.1 will be used for time-to-event endpoints.

Analyses will compare the effect of tirasemtiv on endpoints between the early start and the delayed start treatment groups. Analyses will also be conducted to compare the effect of tirasemtiv within the delayed start treatment group and the early start treatment group, separately. The efficacy endpoints and related analysis sets are listed in Table 3.

Assumptions for statistical models will be graphically evaluated. If assumptions are substantially violated, alternative analysis methods will be considered. Missing data will not be imputed unless specified.

Table 3: CY 4033 Analysis Set and Efficacy Endpoints

Analysis Population	Baseline	Data Included in the Analysis	Efficacy Endpoints	Subgroup Analysis
Full Analysis Set	CY 4031	Including all CY 4031 data (i.e., including data from patients who did and who did not progress into CY 4033) and CY 4033 data	Change from CY 4031 baseline to Week 24 and Week 48 of CY 4033 in percent predicted SVC, ALSFRS-R total score, and ALSFRS-R respiratory domain score	No
			Slope of change from CY 4031 baseline to Week 24 and to Week 48 of CY 4033 in percent predicted SVC, ALSFRS-R total score and respiratory domain score in the analysis comparing the slope change during treatment in the double-blind, placebo-controlled phase in CY 4031 with slope change during treatment with tirasemtiv in the first 48 weeks in CY 4033	No
			Time-to-event endpoints	No
Efficacy Analysis Set	CY 4033	Including CY 4033 data only	Change from CY 4033 baseline to Week 24 and to Week 48 of CY 4033 in percent predicted SVC, ALSFRS-R total score and respiratory domain score	Yes
			Slope of change from CY 4033 baseline to Week 24 and to Week 48 of CY 4033 in percent predicted of SVC, ALSFRS-R total score and respiratory domain score	No
			Time-to-event endpoints	No
Efficacy Analysis Set	CY 4031	Including CY 4031 data only from those patients who completed CY 4031 and progressed into CY 4033 and CY 4033 data	Change from CY 4031 baseline to Week 24 and Week 48 of CY 4033 in percent predicted SVC, ALSFRS-R total score and respiratory domain score	No
			Slope of change from CY 4031 baseline to Week 24 and to Week 48 of CY 4033 in percent predicted SVC, ALSFRS-R total score and respiratory domain score in the analysis comparing the slope change during treatment in the double-blind, placebo-controlled phase in CY 4031 with slope change during treatment with tirasemtiv in the first 48 weeks in CY 4033	No

7.2. Testing Statistical Assumptions Including Comparability at Baseline

Not applicable.

7.3. Subgroup Analyses

Analyses of the efficacy endpoints including change from baseline in percent predicted SVC and in ALSFRS-R total score in subgroups may be conducted on EAS with CY 4033 baseline descriptively. No statistical test will be conducted.

Subgroup factors are:

1. Riluzole Use/Non-Use at the enrollment in CY 4033
2. Gender (Male vs. Female)
3. Age group at Day 1 in CY 4033 (<65, ≥ 65 years old)
4. Geographic region (North America vs. Europe)
5. Time from ALS symptom onset to CY 4031 baseline (< median vs. ≥ median)

TEAEs will be summarized overall in the following subgroups:

1. Gender (Male vs. Female)
2. Age group at Day 1 in CY 4033 (< 65, ≥ 65 years old)
3. Riluzole Use/Non-Use at the enrollment in CY 4033

To graphically display treatment effect changes across subsets, arithmetic mean differences by subgroup level will be produced.

7.4. Multiple Comparisons and Multiplicity

No adjustment will be made for multiple comparisons.

7.5. Analysis of Change from Baseline Endpoints

7.5.1. Change-from-Baseline to Week 24 and to Week 48 in CY 4033

Analyses for the endpoints starting from CY 4031 baseline will be performed on FAS; analyses for the endpoints starting from CY 4033 baseline will be performed on EAS; analyses for the endpoints of change from CY 4031 baseline to Week 48 in CY 4033 will be performed on EAS as well. Please see the analyses of endpoints with related analysis sets in Table 3.

Endpoints of change-from-baseline include change-from-baseline in percent predicted SVC, in ALSFRS-R total score and in ALSFRS-R respiratory sub-score. Descriptive statistics will be provided by visit and by early start and delayed start treatment groups and overall.

The difference in the change from either CY 4031 baseline or CY 4033 baseline at Week 24 or at Week 48 in CY 4033 between patients in the early start and the delayed start treatment (referenced as “treatment” in the model statement below) with tirasemtiv during the open-label extension phase will be evaluated using a repeated-measures mixed model using the restricted maximum likelihood method (Henderson 1984).

The model will include terms for treatment group, percent predicted SVC (or ALSFRS-R) at baseline, visit, pooled site, and CY 4031 baseline riluzole use/non-use as well as interaction terms of treatment-by-percent predicted SVC (or ALSFRS-R) at baseline, treatment-by-visit with an unstructured covariance matrix. If the model fails to converge, other covariance matrix structures (e.g., Toeplitz or compound symmetry) may be used instead of the unstructured covariance matrix. In this case, Akaike's information criterion (proportional to maximized log likelihood – number of parameters) will be used to guide the choice of covariance structure. The least-squares mean of the difference between the early start and the delayed start treatment groups, standard error of the difference, and 95% CIs of the difference will be presented. The treatment effect will be calculated in an estimate statement. The CY 4031 and CY 4033 baselines will be included as a covariate in the different mixed models. For the analyses of change from CY 4031 baseline, data collected from CY 4031 double-blind phase are also included in the model. Section 10 provides sample SAS code for this analysis.

The analyses will be performed on FAS and EAS separately. Treatment group differences will not be estimated if less than one third of the patients have data at Weeks 36 or 48 in CY 4033 in either of the treatment groups and descriptive statistics will be presented. Caution should be used when interpreting estimates when there is extensive missing data. Joint-rank tests or other rank-based analyses may be explored as appropriate. Variable of pooled site will be removed if the model cannot converge in the subgroup analyses.

Least-squares mean (±SE) plots of change-from-baseline at each visit will be provided.

7.5.2. Slope of Changes-from-Baseline

7.5.2.1. The Comparison of Slopes of Changes between the Treatment Groups in CY 4033

Endpoints of slope of changes from baseline include slopes of changes from baseline in percent predicted SVC, in ALSFRS-R total score and in ALSFRS-R respiratory sub-score. Slope of changes from baseline during the first 24 weeks and during the first 48 weeks in CY 4033 will be analyzed using a mixed model which will include early start and delayed start treatment group, baseline value, pooled site, time from baseline, riluzole use/non-use in double-blinded, placebo-controlled phase in CY 4031 as well as interaction terms of early start and delayed start treatment group-by-baseline, early start and delayed start treatment group-by-time, assuming random slope effect. The estimated slope and the slope difference as well as their standard errors will be presented.

Days of change from baseline of the assessments in CY 4033 will be calculated based on the definitions in Section 5.6.1.

The comparisons of slopes of changes from baseline in each parameter are conducted:

1. Slope comparisons of the early start treatment and the delayed start treatment with respect to the estimated rate of change from CY 4031 baseline during the first 24 weeks and during the first 48 weeks in CY 4033
2. Slope comparisons of the early start treatment and the delayed start treatment with respect to the estimated rate of change from CY 4033 baseline during the first 24 weeks and during the first 48 weeks in CY 4033

Scatter plots with regression line fit to the change from baseline either in CY 4031 or in CY 4033 to Week 24 and to Week 48 in CY 4033 will be provided.

7.5.2.2. The Comparison of Slopes of Changes from CY 4031 Baseline During the Treatment Course

The comparison of slopes of changes from CY 4031 baseline during the treatment course will be based on the FAS. A piecewise linear mixed regression model will be used to compare the slope of changes from baseline in CY 4031 to Week 48 in CY 4031 and the slope of changes from baseline in CY 4031 during the first 48 weeks in CY 4033 in the early start and delayed start treatment groups, separately. The model will include the terms of percent predicted SVC (or ALSFRS-R) at baseline value in CY 4031, time from baseline in CY 4031, time from baseline in CY 4033 for the assessments in CY 4033, pooled site, riluzole use/non-use in double-blinded, placebo-controlled phase in CY 4031, assuming random intercept, time from CY 4031 baseline, and time from CY 4033 baseline. The estimated slopes with standard error, difference of slopes between the treatment in double-blind, placebo-controlled phase in CY 4031 and the treatment in the first 48 weeks in CY 4033 in the early start and delayed start treatment groups with standard error and p-value will be presented.

Piecewise regression (Ryan, Porth et al. 2002) line plots of changes from baseline in CY 4031 to Week 48 in CY 4031 and the changes from baseline in CY 4031 to Week 48 in CY 4033 during CY 4033 by treatment group will be provided.

A by-patient listing with demographics, CY 4031 baseline, CY 4033 baseline, clinical visit, assessment date and time, measurement and change from baseline at clinical visits will be presented by patient ID in ascending order and clinical visit date in chronological order within patient.

7.5.3. Sensitivity Analyses of the Change from Baseline

If the percentage of missing values of percent predicted SVC is ≥ 20% at the end of the first 48 weeks in CY 4033, multiple imputation will be used to impute the missing assessments. After the multiple imputations under a missing at random paradigm, imputed values subsequent to death will be set to 50% worse than the values produced from the multiple imputations.

No imputation will be conducted for the missing values of ALSFRS-R.

Subgroup analysis will not be conducted for sensitivity analyses.

7.6. Analysis of the Time-to-Event Endpoints

Analyses for time-to-event endpoints starting from CY 4031 baseline will be performed on FAS; analyses for time-to-event endpoints starting from CY 4033 baseline will be performed on EAS.

Time-to-event endpoints will be summarized using Kaplan-Meier method. A plot of the Kaplan-Meier curves will be provided by early start and delayed start treatment group. Medians, ranges, Q1 and Q3, and corresponding Greenwood's 95% CIs on the treatment estimates will be presented. Patients will be censored at the end of study if the patient does not experience an event prior to date of the last study contact. Log-rank tests (Peto and Peto 1972) will be performed. Cox regression analysis (Cox 1992) will be performed with the fixed factors of early start and delayed start treatment, riluzole use/non-use in double-blinded, placebo-controlled

phase in CY 4031, and percent predicted SVC at baseline, stratifying by pooled site to estimate the hazard ratio and its 95% CI between the early start and the delay start treatment groups with delay start treatment group as reference. The early tirasemtiv start treatment effect will be tested using the likelihood ratio test.

The time-to-event endpoints will be calculated based on the definitions in Section 5.6.1.

Subgroup analysis will not be conducted for time-to-event endpoints.

7.7. Summary of Reasons for Efficacy Non-Evaluability/Exclusion from Efficacy Analyses

Not Applicable.

8. SAFETY AND TOLERABILITY

Analysis of safety and tolerability data will be based on the SAS and will include the time period which extends from the first dose of study medication in Study CY 4033 until the end of the Follow-up period unless otherwise specified. Results will be summarized by early start and delayed start treatment groups and overall.

The analysis of safety and tolerability data includes summaries of tolerability, adverse event preferred terms by system organ class, drug exposure (duration of treatment), dosing information, concomitant medications, clinical laboratory results, vital signs, suicidality assessment, and reasons of early termination from the treatment. Tables summarizing the adverse events reported by patients who died, experienced non-fatal serious adverse events (SAE), or prematurely discontinued the study due to adverse event (AEs) will be prepared. Summaries of potentially clinically notable laboratory results and vital sign abnormalities will be presented. Data listings will be provided to support the summaries of deaths, SAEs, AEs, and laboratory and vital sign abnormalities and will be sorted first by patient then by event preferred term or parameter.

In general, inferential statistical tests are not performed for adverse event incidence rates unless specified.

8.1. Overall Summary of Tolerability

The overall summary of tolerability table will present data by early start and delayed start treatment groups and overall for all treated patients separately. Entries in this table are the number of:

1. Patients treated and days of drug exposure.
2. Patients with treatment emergent adverse events.
3. Patients with serious treatment emergent adverse events.
4. Patients requiring a dose reduction due to adverse events.
5. Patients discontinuing treatment due to adverse events.
6. Deaths.

8.2. Adverse Event Preferred Term and System Organ Class Summary Tables

A treatment-emergent adverse event (TEAE) is an AE with an onset after initiation of study drug dosing in CY 4033, or an AE present at initiation of study drug dosing in CY 4033 that worsens in severity after initiation of study drug dosing.

TEAEs will be assigned to the total daily dose level administered immediately prior to the onset of the TEAE during the CY 4033 treatment.

The Medical Dictionary for Regulatory Activities (MedDRA) will be used to classify Adverse Events by system organ class (SOC) and preferred term (PT). Coding will be performed using version 18.0 of the MedDRA coding dictionary. The severity of adverse events will be evaluated

using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 4.0.

TEAEs will be listed and summarized by the early start and delayed start treatment groups and maintenance dose level as well as total daily dose level administered immediately prior to the onset of the AE. Only TEAEs occurring through 28 days after the last dose of study drug will be summarized. All AEs will be included in patient listings.

8.2.1. Summaries of Adverse Event Incidence Rates for All Patients

Adverse events are graded by the investigator as Grade 1, 2, 3, 4, or 5 according to NCI-CTCAE Version 4.0. The severity grade of events for which the investigator did not record severity will be categorized as "missing" for tabular summaries and data listings. The missing category will be listed last in summary presentation.

The relationship of the event to tirasemtiv and the relationship of the event to riluzole will be collected using the AE eCRF page as follows:

1. AE relationship to tirasemtiv: unrelated, related
2. AE relationship to riluzole: unrelated, related

If the relationship to tirasemtiv is missing, it will be imputed as related to tirasemtiv in the summaries; however, by-patient data listings will show the relationship as missing.

A by-patient listing will include: age, gender dose of tirasemtiv at the onset of the event, time on treatment at the onset of the event, AE onset and resolution dates and times, SAE designation, classification by system organ class and preferred term, the event severity and relationship to study medication, action taken, and outcome sorted by onset date/time and SOC/PT ascendingly.

8.2.2. Missing and Partial AE Onset Dates

The handling of missing and partial AE onset dates are described in the Section 5.7.3 above. Imputed dates will be flagged in the individual patient listings.

8.2.3. Summaries of Adverse Event Incidence Rates for Serious Adverse Events, Adverse Event Dropouts, and Deaths

SAEs and AEs leading to treatment discontinuation or death will also be summarized by system organ class, preferred term, early start and delayed start treatment groups, the total daily dose level administered immediately prior to the onset of the TEAE, and overall. Listings will be presented, for patients who died and/or experienced serious AEs and for patients who discontinued due to TEAEs.

8.3. Total Duration of Therapy, Average Daily Dose, Maximum Daily Dose, Final Daily Dose of Study Medication

8.3.1. Total Duration of Exposure of Tirasemtiv

The total duration of exposure to tirasemtiv will be summarized by early start and delayed start treatment groups and overall for the safety population, regardless of any temporary interruptions in study drug administration, and will be expressed in days using up to 1 decimal place.

Duration of exposure to tirasemtiv in CY 4033 = Last tirasemtiv dose date in CY 4033 - First tirasemtiv dose date in CY 4033 + 1

Duration of exposure to tirasemtiv in CY 4031 and CY 4033 combined for patients randomized to placebo in CY 4031:

Duration of exposure to tirasemtiv in CY 4031 and CY 4033 =

> Last lead-in tirasemtiv dose date in CY 4031 – First lead-in tirasemtiv dose date in CY 4031 +1 + Duration of exposure to tirasemtiv in CY 4033

Duration of exposure to tirasemtiv in Studies CY 4031 and CY 4033 combined for patients randomized to tirasemtiv in Study CY 4031:

Last tirasemtiv dose date in CY 4031 – First lead-in dose date in CY 4031 +1 + Duration of exposure to study drug in CY 4033

When the date of the last tirasemtiv dose is missing or partial, the following rule will be used to impute the last dose date:

- If the last dose date is partial, then: last dose date will be imputed with the earliest of the last date of the month (if month and year are available) or the last month of the year (if only year is available), or the date of death.

The total duration of exposure to tirasemtiv will be summarized using descriptive statistics (number of patients, mean, SD, median, minimum, and maximum) and using the number (i.e., cumulative counts) and percentage of patients exposed through the following time periods for each period in CY 4031 and CY 4033, respectively:

In CY 4031:

Week 4, Week 8, Week 12, Week 16, Week 20, Week 24, Week 32, Week 40, Week 48, Week 56

In CY 4033:

Week 12, Week 24, Week 36, Week 48 and every 12 weeks thereafter.

The number of patients who have down titration or interruption, will be summarized by actual dose level at each clinical visits and phone call in CY 4033.

8.3.2. Average Daily Dose

The total number of tirasemtiv tablets received and average daily dose (mg) in CY 4031 and CY 4033 will be summarized using descriptive statistics (n, mean, SD, median, minimum, and maximum) by early start and delayed start treatment groups.

The total number of tirasemtiv tablets received for patients in CY 4031 and CY 4033 combined will be the sum of the number of tablets collected from first lead-in tirasemtiv dose date in CY 4031 to the last tirasemtiv dose date in CY 4033.

The total number of tirasemtiv tablets received for patients in CY 4033 will be the sum of the number of tablets collected from Day 1 to the last tirasemtiv dose date in CY 4033.

The total amount of tirasemtiv administered (mg) = total number of tablets administered * 125 mg

The average daily dose (mg) will be calculated using the following formula:

$$\text{Average Daily Dose (mg)} = \frac{\text{Total amount of study drug administered (mg)}}{\text{Duration of exposure to study drug}}$$

The maximum daily dose of a patient in CY 4031 and CY 4033 combined is the maximum daily dose from first lead-in tirasemtiv dose date in CY 4031 to the last tirasemtiv dose date in CY 4033. The maximum daily dose of a patient in CY 4033 is the maximum daily dose from Day 1 to the last date of tirasemtiv of the patient in CY 4033.

Final daily dose is the last daily dose administered prior to the study termination in CY 4033.

A separate by-patient listing with the total duration of exposure of tirasemtiv, the total amount of tirasemtiv administered (mg), the average daily dose (mg), maximum daily dose (mg), tirasemtiv accountability will be provided by patient ID (in ascending order) and visit (in chronological order).

8.3.3. Maintenance Dose Level

The maintenance dose level is defined as the scheduled daily dose level at Week 12 in CY 4033. If the patient discontinued the study drug prior to Week 12, the nominal dose level which is close to the average daily dose will be assigned as the maintenance dose.

Week 12 Dose: patients will be assigned to maintenance dose level based on the dose prescribed at their Week 12 visit:

a. 250 mg/day group
b. 375 mg/day group
c. 500 mg/day group

Patients will be assigned to maintenance dose level based on their average dose level defined in Section 8.3.2:

a. 250 mg/day group (0 mg / day ≤ Average dose level < 312.5 mg/day)
b. 375 mg/day group (312.5 mg/day ≤ Average dose level < 437.5 mg/day)
c. 500 mg/day group (Average dose level ≥ 437.5 mg/day)

8.4. Concomitant and Other Medications

Medications other than the study drug reported on the eCRF will be summarized as concomitant medications for the safety population. Medications with an end date that is 7 days or more prior to the first dose of the study drug, or medications with a start date that is 28 days after the last dose of the study drug in CY 4033, will be excluded from the summary. Medications with a start date in CY 4031 and without an end date after the first dose of the study drug in CY 4033, will be included in the summary. The World Health Organization Drug dictionary will be used to classify medications by therapeutic class (ATC Class Level III) and preferred term name. If ATC Class Level III is not available, ATC Class Level II will be used in the summary. Coding will be performed using WHO Drug Dictionary Enhanced Herbal format B2 Mar 2015.

The number and percentage of patients who receive concomitant medication will be summarized by early start and delayed start treatment groups, therapeutic class and preferred term (using ATC Class Level III) and overall. Multiple drug usage by a patient will be counted only once in each category.

In addition, a by-patient listing with start/stop date, days relative to the start of therapy in CY 4033, dose / unit / route / frequency, indication and purpose of all medications taken from Day 1 in CY 4033 or from the first dose in double-blind, placebo-controlled phase in CY 4031 through the end of study will be provided.

8.4.1. Missing and Partial Concomitant and Other Medication Start and Stop Dates

Please see the Section 5.7.2 for the imputation of missing/partial dates of concomitant medication.

Imputed dates will be flagged in the individual patient listings.

8.5. Routine Laboratory Data

Clinical laboratory evaluations, including hematology, serum chemistry, and urinalysis as detailed in the protocol, will be collected at Day 1, Week 4, Week 8, Week 12 and every 12 weeks thereafter, discontinuation clinical visit and at the Follow-up Visit. Descriptive statistics for laboratory assessment values and their changes from baseline will be presented at each assessment time point by early start and delayed start treatment, maintenance dose level and overall. Baseline is defined as the last non-missing assessment value prior to the first dose of study drug in CY 4033. In the case of multiple values in an analysis window defined in Section 5.6.6, data will be selected for analysis as described in Section 5.6.7.

The number and percentage of patients with abnormal laboratory values will be presented by early start and delayed start treatment groups, maintenance dose level and overall. The lower limit of normal and upper limit of normal provided by the laboratories will be the criteria used to determine abnormal laboratory values. The number and percentage of patients with potentially clinically significant (PCS) abnormal laboratory values will be presented by early start and delayed start treatment groups and overall. Severe PCS abnormal laboratory values are defined as Grade 3 laboratory abnormalities according to NCI-CTCAE Version 4.0.

In calculating percentages of patients with abnormal laboratory values for each parameter, the denominator is the number of dosed patients with a normal or missing baseline assessment and with at least one post-baseline assessment (patients with an abnormal baseline laboratory value will be excluded from the denominator for that parameter); the numerator is the number of dosed patients with a normal or missing baseline assessment and at least one abnormal post-baseline assessment (including unscheduled assessments, and assessments at the Follow-up Visit) at the treatment group and overall.

The boxplots will be presented for selected laboratory quantitative tests over time in CY 4033.

A shift table will be provided in the form of shifts from baseline NCI-CTCAE grade to the maximum grade post baseline by early start and delayed start treatment groups, laboratory tests. Shifts for the last available on-drug assessments and at the follow-up visit also will be included. Only laboratory tests with available NCI-CTCAE toxicity grade reference ranges will be included.

A by-patient listing of clinical laboratory (hematology, serum chemistry, urinalysis, and other) with demographic, visit, the actual assessment date and time, relative day, type of visit, laboratory test, lab test units, lab test results, and the lower and upper limits of normal sorted by patient ID and chronologically by visit, the actual assessment date and time will be presented. Values outside the Laboratory's normal ranges will be flagged. Unscheduled laboratory values also will be noted.

The association between concentration of tirasemtiv and the change from baseline in selected laboratory tests may be explored.

8.6. Vital Signs

Vital signs, which include heart rate, respiration rate, blood pressure measured at rest (i.e. after the patient sits or has been supine for at least 3 minutes), and weight (kg) will be obtained at Day 1, Week 4, Week 8, Week 12 visits and every 12 weeks visits thereafter, at the discontinuation clinical visit and at the 28 Day Safety Follow-up Visit. Height (cm) will be collect at Screening visit of CY 4031. Weight (kg) will be obtained at each clinic visit starting from the Screening through the Follow-up Visit in CY 4033.

Descriptive statistics (n, mean, SD, median, minimum, and maximum) of observed values and changes from CY 4033 baseline of vital sign parameters will be presented at each assessment time point and by early start and delayed start treatment groups and overall. Repeat and multiple measurements will be processed analogously with the method described in the clinical laboratory data in Section 8.5.

The number and percentage of patients with PCS vital signs, using the criteria specified in Table 4, will be presented by the early start and delayed start treatment groups and overall. For each parameter, the denominator is the number of patients with non-PCS or non-missing baseline assessment and with at least one post baseline assessment; and the numerator is the number of patients with non-PCS or non-missing baseline assessment and with at least one post baseline PCS value including repeated and unscheduled measurements (subset of the denominator). Assessment at Follow-up Visit will also be included in the summary.

Descriptive statistics of weight and weight loss from CY 4031 baseline and from CY 4033 baseline will be presented by visit and by early start and delayed start treatment groups. Scatter plots with regression line fit to the weight loss from baseline either in CY 4031 or in CY 4033 will be presented.

A by-patient listing of individual vital signs with demographic, visit, the actual assessment date and time, relative day, type of visit, and vital sign values with units sorted by patient ID and chronologically by visit, the actual assessment date and time will be presented. PCS vital signs will be flagged. Unscheduled vital sign values also will be noted. Table 4 below lists the criteria for clinically significant values outside of normal range of vital signs.

Table 4: Criteria for Clinically Significant Vital Signs

Vital Sign Parameter	Flag	PCS Criteria
Systolic Blood Pressure (mmHg)	High	≥160 mmHg
	Low	≤80 mmHg
Diastolic Blood Pressure (mmHg)	High	≥100 mmHg
	Low	≤ 50 mmHg
Respiration Rate (Breaths per minute)	High	>18 breaths per minute
	Low	<8 breaths per minute
Pulse (beats per minute)	High	≥120 bpm
	Low	≤50 bpm
Weight (kg)	Clinically Significant	≥5% reduction from baseline

8.7. Suicidality Assessment

The suicidality assessment (Beck Depression Inventory (BDI) Fast Screen) will be collected at the Day 1, Week 4, Week 8, Week 12 clinical visits and every 12 weeks thereafter, Tirasemtiv Discontinuation Clinic Visit, Tirasemtiv Discontinuation Phone Call, and at the 28 Day Safety Follow-up Clinic Visit/Phone Call in CY 4033.

The BDI total score is the sum of the scores of the seven individual questions, where each question is scored from 0 to 3. The total score of the BDI cannot be calculated if answer to any of the seven individual questions is missing.

Total BDI scores at each assessment will be classified as shown in Table 5.

Table 5: Depression Level Classification by BDI Total Score

Depression Level	BDI Total Score
Minimal Depression	0 - 3
Mild Depression	4 - 6
Moderate Depression	7 - 9
Severe Depression	10 - 21

A by-patient listing of individual BDI score with demographic, visit week, actual assessment date, relative day, and total score with depression classification sorted by early start and delayed start treatment groups, and chronologically by visit week, the actual assessment date will be presented. The worst values of the total score and an individual question, Suicidal Thoughts, for each patient will be flagged.

8.8. Physical Examination

Physical examination will not be listed.

8.9. Study Termination Status

Number and percentage of patients who complete and prematurely discontinue planned study dosing with reasons for premature discontinuation will be included in the disposition table.

9. PHARMACOKINETIC/ PHARMACODYNAMIC ANALYSIS

9.1. Pharmacokinetic Analysis

Pharmacokinetics (PK) analyses will be based on the PK analysis set. Concentration data for tirasemtiv will be summarized by early start and delayed start treatment groups by nominal sampling time using descriptive statistics (arithmetic mean, SD, median, minimum, maximum, geometric mean, and coefficient of variation). All concentrations below the lower limit of quantification (LLOQ) will be set to zero for purposes of calculating descriptive statistics.

The trough concentration will be modeled as a function of body weight over time using a repeated measurement mixed model, including early start and delayed start treatment group, clinical visit, gender and age group (<65 and ≥65 years old) as covariates.

The concentration will be taken as the natural log-transformation before using the analysis in the models and the back-transformation (geometric mean ratio) and back-transformed 90% CI will be reported.

A by-patient listing with demographic, treatment group, visit week, study drug administration date and time, sample collection data and time, relative day, and PK time point, PK concentration and LLOQ of tirasemtiv sorted by early start and delayed start treatment groups, patient ID and chronologically by visit week, the actual assessment date and time will be presented.

Concentrations of riluzole will not be analyzed in this study.

9.2. Pharmacodynamic Analysis/ Pharmacokinetic Analysis

Pharmacodynamic (PD) analyses will be performed to graphically explore the relationship between trough tirasemtiv concentration and efficacy and safety endpoints by early start and delayed start treatment groups based on the PD Analysis Set.

Changes from baseline in PD measurements will be paired with coincidentally measured trough plasma concentrations of tirasemtiv. Only the concentration measurements collected within the time window around the nominal time as per the analysis specifications will be included in the analyses based on concentration. For all these parameters, baseline is defined as the last non-missing assessment, including unscheduled assessments, made prior to the initial dose at Day 1 in CY 4033. The PD assessments will include:

- SVC
- ALSFRS-R total score and respiratory sub-score
- Weight loss

10. STATISTICAL CODES

The following code will be used as the prototype of the codes that will be used for the final analysis of the study. The final version of the statistical codes to be used will be determined and will be documented in the specification document for the statistical report of the study.

1. SAS code for the final analysis for the efficacy endpoint at Week 24 and Week 48

```
proc mixed data=work;
class <subject> <riluzole use> <pooled site> <dose level> <treatment> <visit>;
model chg=<base>  <riluzole use> <visit> <pooled site> <treatment> <treatment>*<base>
<treatment>*<visit> /solution ddfm=kr ;
repeated <visit>/subject=<subject> type=un;
lsmestimate <treatment>*<visit>
'Early-start vs. Delayed-start at Week 24' [-1,1 4] [1,2 4]/ e cl elsm;

ods output LSMEstimates=lsme ;
run;
```

Variable VISIT has four categories with Day 1 coded as 1 and Week 24 coded as 4.

Analysis at Week 48 will use a similar model where the level for VISIT is 6 for Week 48.

```
'Early-start vs. Delayed-start at Week 48' [-1,1 6] [1,2 6]/e cl elsm;
```

2. SAS code for slope analysis between treatment group during CY 4033

```
PROC MIXED data=work method=REML;
class <Subject> <treatment> <pooled site> <riluzole use>;
model <chg> = <base> <treatment> <pooled site> <days from base> <riluzole use>
<treatment>*<base>  <treatment>*<days from base>/
ddfm=kr noint;
Random <days from base> / type=un subject=<Subject>;
Estimate 'Slope Diff between pooled early-start vs. delayed-start' <treatment>*<days
from base>  1 -1/e cl;
run;
```

Change from baseline at baseline time point (change=0) is included in the input dataset and no intercept is fit to the model.

3. SAS code for slope analysis during the clinical course of treatment

```
proc mixed data=work method=REML;
    class  <subject> <pooled site> <riluzole use>;
    model chg=<base> <riluzole use> <pooled site> <days from CY 4031 base> <days from
CY 4033 base> <base> * <days from CY 4031 base>    /s ddfm=kr;
    random intercept <days from CY 4031 base> <days from 4033 base> / sub=<subject>
type=un g gcorr;
      estimate 'Slope in CY 4031: Placebo '    <days from CY 4031 base 1 <day from
CY 4033 base > 0 /e cl;
      estimate 'Slope in CY 4033: Placebo '    <days from CY 4031 base 1 <day from
CY 4033 base > 1 /e cl;
      estimate 'Slope Difference between in CY 4033/CY 4031: Placebo '    <days from
CY 4031 base 0 <day from CY 4033 base > 1 /e cl;
run;
```

Coding: `<days from CY 4031 base>` is the days from CY 4031 baseline time point (change=0)

<days from CY 4033 base> is the days from CY 4031 baseline time point of the assessments in CY 4033 only. If the assessments in CY 4031, the values will be assigned to 0.

4. The SAS code for proportional hazard Cox regression model for time to onset endpoints is as follows.

```
PROC phreg data=work;
class <treatment>(ref=first) <pooled site> <Riluzole use>;
model <response>*censor (<censor list>)=<SVC base> <treatment> <riluzole use>;
hazardratio <treatment>;
strata <pooled site>;
Run;
```

5. The SAS code for multiple imputations is as follows.

Step 1: Impute the missing data

```
PROC mi data=work seed=13113 nimpute=50 out=miwork;
mcmc chain=multiple;
var <day 1> <week 4> <week 8> <week 12> <week 24> <week 36> <week 48>;
by <treatment>;
run;
```

Step 2: Analyze the complete data

```
PROC MIXED data=miwork METHOD=REML;
class <Subject> <Treatment> <Visit> <pooled site> <Riluzole use>;
model <chg> = <Baseline> <Baseline>*<Visit> <Treatment> <Visit> <pooled site>
<Riluzole use> <Treatment>*<Visit>/
ddfm=kr solution covb;
repeated <Visit> / SUBJECT=<Subject> Type=UN;
lsmestimate <treatment>*<visit> 'Early-Start vs. Delayed-start at Week 24'

[-1,1 4] [1,2 4]
/e cl elsm;
ods output LSMEstimates=est;
By _imputation_;
run;
```

Step 3: Combine the results of the analysis of imputations.

```
PROC Mianalyze data=est;
Modeleffects estimate;
Stderr stderr;
Run;
```

6. The normality assumptions for the ANCOVA analysis will be assessed by residual illustration. The outpred=outpred option in in the model statement in the above MIXED model stores residuals which are used to test the assumption of normality. Examination of residuals can be done using the following codes.

```
PROC UNIVARIATE DATA=OUTPRED NORMAL PLOT;
VAR RESID;
QQPLOT RESID;
RUN;

PROC PLOT DATA=TEMP;
```

```
PLOT RESID*PRED;
RUN;
```

7. The SAS code for Kaplan-Meier method is as follows:

```
PROC LIFETEST data=AE;
time <Var_time>*dizziness(0);
strata <treatment>;
run;
```

where Var_time is either time to the first onset of dizziness or duration of the first dizziness under each treatment/dose level (total daily dose).

8. The following SAS code will be used in evaluation of the associations between observed concentration and the time to the onset of dizziness:

```
PROC PHREG data=work;
model <time_to_event>*<censor>(0) = PK parameter of interest /rl;
id patient;
Run;
```

9. The SAS code to evaluate concentration effect expressed in terms of slope estimated from ANCOVA models is as follows:

```
PROC MIXED data=work METHOD=REML;
Class <Subject> <Riluzole use> <pooled site> <visit>;
Model <chg> = <Baseline> <pooled site> <concentration> /ddfm=kr;
Repeated <visit> /type=UN subject=<Subject>;
run;
```

11. REFERENCES

Beck, A. T., R. A. Steer, et al. (1996). "Beck depression inventory-II." San Antonio **78**(2): 490-498.

Cedarbaum, J. M., N. Stambler, et al. (1999). "The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III)." J Neurol Sci **169**(1-2): 13-21.

Cox, D. R. (1992). Regression models and life-tables. Breakthroughs in statistics, Springer**:** 527-541.

Henderson, C. (1984). "Applications of linear models in animal breeding (University of Guelph, Guelph, ON, Canada)."

Kalbfleisch, J. D. and R. L. Prentice (2011). The statistical analysis of failure time data, John Wiley & Sons.

Kaplan, E. L. and P. Meier (1958). "Nonparametric estimation from incomplete observations." Journal of the American statistical association **53**(282): 457-481.

Knudson, R. J., M. D. Lebowitz, et al. (1983). "Changes in the normal maximal expiratory flow-volume curve with growth and aging." Am Rev Respir Dis **127**(6): 725-734.

Peto, R. and J. Peto (1972). "Asymptotically efficient rank invariant test procedures." Journal of the Royal Statistical Society. Series A (General): 185-207.

Ryan, S. E., L. S. Porth, et al. (2002). "Defining phases of bedload transport using piecewise regression." Earth Surface Processes and Landforms **27**: 971-990.

APPENDIX 1. SCHEDULE OF EVENTS

Procedures	Day 1 Clinic	Week 2 Call	Week 4 Clinic	Week 6[3] PI/Sub-I Call	Week 8 Clinic	Week 10 Call	Week 12 Clinic	Week 24 Clinic	Week 36 Clinic	Week 48 Clinic	Every 12 Weeks Ongoing Clinic	*Tirasemtiv* Disc Clinic Visit	*Tirasemtiv* Disc Phone Call	28 Day Safety Follow-up Clinic Visit	28 Day Safety Follow-up Phone Call
Informed Consent	X														
Inc/Exc Criteria	X														
Demographic Data	X														
Physical Examination	X														
Concomitant Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X		X		X		X	X	X	X	X	X		X	
Clinical Safety Labs[1]	X		X		X		X	X	X	X	X	X		X	
Serum Pregnancy Test[2]	X							X		X		X[2]			
PK Sample[4]							X	X		X		X		X	
SVC	X		X		X		X	X	X	X	X	X		X	
ALSFRS-R	X		X		X		X	X	X	X	X	X	X	X	X
Suicidality Assessment	X		X		X		X	X	X	X	X	X	X	X	X
AE Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tirasemtiv Dosing	X	X	X	X	X	X	X	X	X	X	X				

1 TSH every year after Day 1 Clinic Visit

2 Serum pregnancy test only for females of child bearing potential at Day 1 Clinic Visit, every 24 weeks thereafter, and at the *Tirasemtiv* Discontinuation Visit

3 Phone contact with patient by PI or designee with prescriptive authority in their local jurisdiction

4 PK Sample to be collected every 24 weeks after the Week 48 Clinic Visit as well as *Tirasemtiv* Discontinuation and 28 Day Safety Follow-Up Visits

Signature Manifest

Document Number: PRD-0009 Revision: 00

Title: CY 4033 - Statistical Analysis Plan (A Phase 3, Open-Label Extension Study of Tirasemtiv for Patients with Amyotrophic Lateral Sclerosis (ALS) who Completed VITALITY-ALS (CY 4031))

Effective Date: 09 Nov 2017

All dates and times are in Pacific Time.

CY 4033 SAP

1: Electronic Approvals

Name/Signature	Title	Date	Meaning/Reason
Long Ma (LMA)	Sr. Biostatistician	09 Nov 2017, 10:05:40 AM	Approved
Lisa (Lixin) Meng (LMENG)	Sr. Director, Biometrics	09 Nov 2017, 10:29:28 AM	Approved
Bettina Cockroft (BCOCKROFT)	VP, Clinical Research, Neuro	09 Nov 2017, 10:57:35 AM	Approved