# STATISTICAL ANALYSIS PLAN

**VERSION: 2.0**

**DATE OF PLAN: 26-JUN-2017**

**PROTOCOL NUMBER: CY 4031**
**BASED ON PROTOCOL DATED JUNE 26, 2017**

**STUDY DRUG: *TIRASEMTIV***
(Formerly known as CK-2017357)

**STUDY TITLE:**

A Phase III, Multi-National, Double-Blind, Randomized, Placebo-Controlled, Stratified, Parallel Group, Study to Evaluate the Safety, Tolerability and Efficacy of *Tirasemtiv* in Patients with Amyotrophic Lateral Sclerosis (ALS)

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# REVISION CHRONOLOGY

February 3, 2016 Original, Version 1.0

June 26, 2017 Version 2.0 (Amendment 1)

Section 4.1.1 (Full Analysis Set (FAS)). The definition of the Full Analysis Set (FAS) was updated to clarify the requirement that all patients have at least one "post randomization" efficacy assessment.

Section 4.2 (Use of Analysis Sets in Different Analyses). Table 3 displays the analysis datasets used by analyses was updated. Demographics and baseline characteristics summary tables will be provided for the FAS, Per Protocol Analysis Set (PPS) and Safety Analysis Set (SAS). Efficacy analyses will be provided for the FAS and PPS. A statement that analyses based on the PPS will not be repeated if the number of patients in the PPS is ≥ 90% of the number of patients in the FAS.

Section 4.3 (Pooled Sites) and Appendix B (Table of the Regional Site Pooling for FAS). The final plan for pooling of study sites was added.

Section 4.4.1 (Subgroup Efficacy Analyses). This section was modified to

- Clarify that subgroup analyses are to be performed for the primary efficacy endpoint and secondary endpoints evaluating the change from baseline in the ALSFRS-R respiratory domain score at the end of 48 weeks of the Double-Blind, Placebo-Controlled Phase and the slope of muscle strength mega-score during the 48 weeks of the Double-blind, Placebo-Controlled Phase. The same modifications were made to Section 7.2.7 (Subgroup Efficacy Analyses) for consistency.
- Remove duplicate analyses across sections. The following statement was removed because it is also specified in Section 8 Safety Analyses: "Safety signals will be examined by riluzole use/non-use. Additional subgroups for exploratory safety analyses may be identified after the planned safety analyses have been conducted."
- Specify that subgroup analyses will be performed using the FAS only. The objective of subgroup analyses is to evaluate the consistency of primary and the two secondary endpoint results across subpopulations. The PPS has been removed, as it is a subset of the full analysis set (FAS).
- Add two subgroups: a) time from symptom onset (<median, ≥median) and b) patients with scores of 2 or more on all items of ALSFRS-R at baseline, percent predicted SVC ≥ 80% at baseline, definite or probable ALS, and time from symptom onset ≤ 24 months. These patients subsets are added because of their general interest to ALS clinical researchers.
- Remove two subgroups: a) slow progressors vs. rapid progressors in SVC change identified using the empirical cut-off of slope from baseline to Month 6 visit based on EMPOWER natural history data (< median, ≥ median) and b) rate of weight loss from baseline to Week 24 (< median, ≥ median). The definitions of the two subgroups are based on information collected after patients started Double-Blind, Placebo-Controlled Phase which would be confounded with the treatment effect to be evaluated.

Section 4.4.1.1 (Maintenance Dose Group Definitions). This section was added to specify the procedures for assignment of patients to treatment groups based on their maintenance dose. Two methods for determining the maintenance dose are included.

Section 6.4 (Medication History and Concomitant Medication). The dictionary version of WHO DRUG was updated to reflect the version used in the clinical database.

Section 7.1.2 (Secondary Endpoints) and Section 7.2.9 (Multiplicity). The secondary endpoint based on the respiratory components of the ALSFRS-R has been redefined, procedures for testing the secondary endpoints have been modified, and references to exploratory testing for differences by randomized target dose group have been deleted from Section 7.2.9:

- Time to the first occurrence of a decline in any of the respiratory components of the ALSFRS-R (i.e., items 10, 11, and 12) or death during the 48 weeks of Double-Blind, Placebo-Controlled Phase was replaced by the change from baseline in ALSFRS-R respiratory domain score at the end of 48 weeks of the Double-Blind, Placebo-Controlled Phase. The rationale for changing the endpoint is to avoid uncertainty regarding handling events that occur during the Open-Label Phase and also to avoid potential confounding by early events that could exclude many more subsequent months of data during the Double-Blind, Placebo-Controlled Phase from consideration.
- Secondary endpoints that are based on SVC have been moved to the end of the hierarchy, along with the endpoint time to the first use of mechanical ventilatory assistance or death. Secondary endpoints based on time to changes in SVC would contribute only incrementally to a greater understanding of the potential beneficial effect of *tirasemtiv* beyond that provided by the SVC primary endpoint (change in SVC at 24 weeks). Change from baseline in ALSFRS-R Total score at the end of 48 weeks was added after the secondary endpoints based on SVC because of its general interest to ALS clinical researchers. Time to the first use of mechanical ventilatory assistance or death has been moved to the end of hierarchy due to the low number of such events through 48 weeks of follow-up.
- If the null hypothesis for the primary endpoint is rejected, the Benjamini-Hochberg method will be used to simultaneously test the change from baseline in the ALSFRS-R respiratory domain score and the slope of muscle strength mega-score during the 48 weeks of the randomized, Double-Blind, Placebo-Controlled Phases.
- Testing for differences from placebo by randomized dose group have been deleted in response to comments made by FDA on the statistical analysis plan version 1.0 [Comments received via email on April 15, 2016].

Section 7.1.3 (Tertiary Endpoints). Proportion of patients whose decline from baseline in the ALSFRS-R respiratory domain score is $\leq 2$ at the end of 48 weeks was added as a tertiary endpoint. Item number 4 was modified to include the slope of muscle strength mega-score during the first 24 weeks of Double-Blind, Placebo-Controlled Phase and to exclude the slope of change from baseline to 48 weeks in muscle strength mega-score from the tertiary endpoints. ALSFRS-R total score, ALSFRS-R respiratory domain score were removed from item number 5.

Modifications in the item number 4 and 5 are due to the updates made in Section 7.1.2 Secondary Endpoints.

Section 7.2.1 (Primary Analysis). "Week 24" was added in the last sentence of this section. This modification was made in response to comments from the FDA [Comments received via email on April 15, 2016] to clarify that 5% missing data refers to Week 24 at which time point the primary analysis of the primary endpoint is performed.

Section 7.2.2 (Secondary and Tertiary Efficacy Analyses). Assessments of the relationship between change from baseline in percent predicted SVC and change from baseline in the ALSFRS-R respiratory domain score at Week 24 and Week 48 were added.

Section 7.2.5 (Supportive Analyses). This section was modified to be consistent with the statistical analysis section of the protocol (Section 8.4.4) that identifies the rank based analysis as a supportive analysis. Criteria specifying when within-visit rank analyses will be performed were added. These modifications were made in response to FDA comments [Comments received via email on April 15, 2016] on the Statistical Analysis Plan version 1.0.

Section 7.2.6 (Sensitivity Analysis). Item number 6 was modified to clarify events that will be included in a multiple time to events analysis. Modification was also made to the statistical method used to analyze the time to multiple events. These modifications were made in response to FDA comments [Comments received via email on April 15, 2016] on the Statistical Analysis Plan version 1.0.

Section 8 (Safety Analyses). Safety analyses for the first 24 weeks of the Double-Blind, Placebo-Controlled Phase were removed. Safety analyses for the first 24 weeks of the Double-Blind, Placebo-Controlled Phase were part of a planned final analysis of the primary endpoint prior to completion of the study in Protocol Amendment 01 (dated 31 July 2015). Safety analyses for the first 24 weeks were deleted, because the final analysis of the primary endpoint will be conducted after all patients completed the study (see Protocol Amendment 02, dated 16 January 2016).

Section 8.2.1.2 (Definition of TEAEs in the Double-Blind, Placebo-Controlled of the Study) was modified to add two alternative definitions of TEAEs in the 48 weeks of the Double-Blind, Placebo-Controlled Phase and the *Tirasemtiv* Withdrawal Phase. Summarizing TEAEs based on each of the three approaches for assigning AEs that persist from the Open-Label Phase into the Double-Blind, Placebo-Controlled Phase (i.e., "exclusionary," "prevalence," and "incidence" approaches) to the patients' randomized target dose group should help to assess the AE profile of *tirasemtiv* relative to placebo. See Section 8.2.1.2 for a description of the rationale for the additional alternatives definitions of TEAEs.

Section 8.2.2 (TEAE summaries) was modified to add summaries of TEAEs across all three study phases (i.e., the Open-Label Phase, the Double-Blind, Placebo-Controlled Phase, and the *Tirasemtiv* Withdrawal Phase, including the follow-up period). Clarification of dose level assignment for TEAEs in the Double-Blind, Placebo-Controlled Phase was also made.

Section 8.2.4 (Time to First Onset and Duration of Dizziness). Clarifications of the time to onset of dizziness at the time of first dose of double-blind study medication for different scenarios were made. The analysis of evaluating the associations between the *tirasemtiv* plasma concentration and the time to the onset of dizziness was removed because PK sampling is sparse

and *tirasemtiv* plasma concentrations are relevant when the onset of dizziness is not on the same date of the scheduled PK blood sampling. An analysis evaluating the association between the *tirasemtiv* plasma concentrations and the presence of dizziness on Day 1 and at Week 4/Early Termination prior to Week 4 visits was added.

Section 8.3 (Clinical Laboratory Evaluations). This section was modified to add shift tables from baseline NCI-CTCAE grade to the maximum grade post randomization for each study phase for randomized and dosed patients. A separate listing of patients who reported post randomization CTCAE toxicity grade ≥3 laboratory rest results was added.

Section 8.4 (Vital Signs). Shift tables were added for the randomized and dosed patients from baseline PCS classification to post randomization PCS classification.

Section 9.1 (Pharmacokinetic Analyses). This section was modified to correct the omission of *tirasemtiv* in addition to *tirasemtiv* metabolites, to remove analysis comparing riluzole levels in presence and absence of *tirasemtiv* and only summarize PK levels using descriptive statistics. The effect of *tirasemtiv* on riluzole PK will be assessed in a separate population PK analysis combining data from all studies conducted with *tirasemtiv* in patients with ALS.

Section 9.2 (Pharmacokinetic/Pharmacodynamic Analyses). This section was modified to simplify and make more general the language describing the PK/PD analyses.

Section 10.2.3 (Time to Event Endpoints). The second censoring scenario was removed. Patients are encouraged to come back for the remaining study clinical visits after early termination from study treatment. Censoring by the time of early termination of the study treatment approach does not include information collected during the study and therefore was removed.

Section 10.2.4 (Responder Endpoints). Item 1 was modified to clarify the responder definition for patients who do not complete the study. An editorial error was corrected in item 2 to clarify that completers are patients who are on study treatment for at least ¾ of the time.

Section 10.2.8 (Skeletal Muscle Strength). This section was modified to remove the step to exclude muscle groups with baseline strength greater than the mean strength in the normal subject population or in the top 10% of strength achieved by all patients to avoid a ceiling effect. Because few muscle groups are expected to remain normal during 48 weeks of treatment in the Double-Blind, Placebo-Controlled Phase, there is no need to consider ceiling effect in this study.

Section 10.3 (Analysis Visit Windows). This section was modified to clarify when the analysis visit windows will be applied. Clarification was also made for the analysis visits used for the safety assessment performed at scheduled clinical visits.

Editorial Modifications:

References to the “randomized treatment groups” were modified to use ‘randomized target dose group’ throughout the document.

Dose level definition clarifications were added to Sections Section 8.2.2, 8.3 , 8.4 , 8.5 , 8.6, 8.7 and Section 8.8.

The analysis sets in the following sections were updated, Section 6.1, 6.3, and Section 6.4. The analysis sets in these sections were updated to be consistent with the updates made to Section 4.2. Other editorial corrections were made throughout.

# LIST OF ABBREVIATIONS

| Abbreviation | Term |
|---|---|
| AE(s) | Adverse event(s) |
| ALS | Amyotrophic lateral sclerosis |
| ALSFRS-R | ALS Functional Rating Scale-Revised |
| ANCOVA | Analysis of Covariance |
| ANOVA | Analysis of Variance |
| BID | Twice a day |
| BMI | Body mass index |
| Bpm | Breaths per minute |
| CMH | Cochran-Mantel-Haenszel |
| CI | Confidence interval(s) |
| CRF/eCRF | Case report form/electronic case report form |
| DMC | Data Monitoring Committee |
| ECG | Electrocardiogram |
| EDC | Electronic data capture |
| FAS | Full analysis set |
| GEE | Generalized Estimating Equations |
| HHD | Hand-held dynamometry |
| kg | Kilogram |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mg | Milligram |
| mL | Milliliter |
| Mm | Millimeter |
| mmHg | Millimeters of mercury |
| NCI-CTCAE | National Cancer Institute Common Terminology Criteria for Adverse Events |
| NCS | Not Clinically Significant |
| ng | Nanogram |
| NSS | Non-self-sufficiency |
| PCS | Potentially Clinically Significant |
| PD | Pharmacodynamic(s) |
| PK | Pharmacokinetic(s) |

| Abbreviation | Term |
|---|---|
| PKEDS | Pharmacokinetic evaluable data set |
| PPS | Per protocol set |
| SAP | Statistical Analysis Plan |
| SAS | Safety Analysis Set |
| SAS® | Statistical Analysis System |
| SD | Standard deviation |
| SNIP | Sniff Nasal Inspiratory Pressure |
| SVC | Slow Vital Capacity |
| $T_{max}$ | Time of maximum plasma concentration |
| TEAE(s) | Treatment-emergent adverse event(s) |
| WHO | World Health Organization |

# 1. INTRODUCTION

This statistical analysis plan (SAP) provides a technical and detailed elaboration of the planned statistical analyses of CY 4031 data. It is based on Protocol Amendment 03 dated June 26, 2017. Any additional analyses performed after unblinding will be deemed exploratory.

CY 4031 is a multi-national, double-blind, randomized, placebo-controlled, stratified, parallel group study of the selective fast skeletal muscle troponin activator, *tirasemtiv*, in patients with ALS who can complete two weeks of treatment with open-label *tirasemtiv* (125 mg twice a day [BID]). Patients who are currently taking riluzole and those who are not will be enrolled in the study. Patients taking riluzole who are randomized to *tirasemtiv* will have their riluzole dose decreased to 50 mg/day (half the approved dose) in a double-blind fashion, since previous studies have demonstrated that administration of *tirasemtiv* approximately doubles the exposure to concomitant riluzole.

The study includes three phases: an Open-Label Phase, a Double-Blind, Placebo-Controlled Phase, and a Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase.

Following completion of two weeks of treatment with open-label *tirasemtiv*, patients will be randomized 3:2:2:2 to placebo and three different target total daily dose levels of *tirasemtiv,* 250 mg, 375 mg and 500 mg for 48 weeks. Table 1 summarizes dosing schedule for each treatment group.

**Table 1: Dosing Schedule by Treatment Group**

| **Group 1 (Placebo)** | **Group 2 (target 250 mg/day of *tirasemtiv*)** | **Group 3 (target 375 mg/day of *tirasemtiv*)** | **Group 4 (target 500 mg/day of *tirasemtiv*)** |
|---|---|---|---|
| Weeks 1 through 48<br>2 placebo tablets twice daily | Weeks 1 through 48<br>1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in AM and 1 tablet of *tirasemtiv* (125 mg) and 1 tablet of matching placebo in PM | Weeks 1 and 2<br>1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in AM and 1 tablet of *tirasemtiv* (125 mg) and 1 tablet of matching placebo in PM<br><br>Weeks 3 through 48<br>1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in AM and 2 tablets of *tirasemtiv* (250 mg) in PM | Weeks 1 and 2<br>1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in AM and 1 tablet of *tirasemtiv* (125 mg) and 1 tablet of matching placebo in PM<br><br>Weeks 3 and 4<br>1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in AM and 2 tablets of *tirasemtiv* (250 mg) in PM<br><br>Weeks 5 through 48<br>2 tablets (250 mg) of *tirasemtiv* in AM and 2 tablets of *tirasemtiv* (250 mg) in PM |
| Riluzole Users Only | | | |
| Group 1<br>(Placebo) | Groups 2-4<br>(Active Treatment Groups) | | |
| Riluzole tablets:<br>50 mg riluzole (personal supply) in the morning plus blinded riluzole 50 mg in the evening | Riluzole tablets:<br>50 mg riluzole (personal supply) in the morning plus blinded riluzole placebo in the evening | | |

Upon completion of the Double-Blind, Placebo-Controlled Phase, patients receiving active treatment will be randomized a second time to placebo or to continue their current dose level of active treatment in an allocation ratio of 1:1 for the next four weeks as part of the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase. Patients who are on placebo will be assigned to placebo at the end of Week 48. Both randomizations will be stratified by riluzole use/non-use. A schedule of study procedures is given in Appendix A.

# 2. OBJECTIVES

## 2.1. Primary Objective

The primary objective is to assess the effect of *tirasemtiv* versus placebo on respiratory function in patients with ALS.

## 2.2. Secondary Objectives

Secondary objectives include:

- Evaluation of alternative methods to assess the effect of *tirasemtiv* versus placebo on percent predicted slow vital capacity (SVC) in patients with ALS
- Assessment of the effect of *tirasemtiv* versus placebo on other clinical measures related to the progressive decline in respiratory function in patients with ALS
- Assessment of the effect of *tirasemtiv* versus placebo on other measures of skeletal muscle function in patients with ALS

# 3. SAMPLE SIZE

Approximately 600 patients will be enrolled in the study. Following the open-label phase, approximately 477 patients who can tolerate the two weeks of open-label 125 mg BID *tirasemtiv* will be randomized to placebo and three different target dose levels of *tirasemtiv* in an allocation ratio of 3:2:2:2 for the Double-Blind, Placebo-Controlled Phase. The dropout rates at 24 weeks are estimated to be 16% for placebo and 25% for all *tirasemtiv* target dose groups combined. With a two-tailed alpha error of 0.05, approximately 360 patients are expected to complete 24 weeks of double-blind treatment, which is estimated to provide 90% power to detect a treatment difference from placebo in percent predicted SVC change from baseline to the end of the first 24-week Double-Blind, Placebo-Controlled Phase of 6 percentage points for all *tirasemtiv* target dose groups pooled with a common standard deviation (SD) of 17 percentage points.

The common SD of the primary endpoint (the change from baseline to Week 24 of the Double-Blind, Placebo-Controlled Phase in percent predicted SVC) will be monitored in a blinded manner. When one-half of the planned completed study patients have been followed for 24 weeks, the common SD of the primary endpoint will be calculated on aggregate data with no separation of results by treatment group. If this common SD during the study appears to be larger than 17 percentage points, the sample size will be re-estimated with the updated common SD to maintain the intended study statistical power for the primary analysis using the table below. For example, if the common SD is larger than 17 and equal to or less than 18, then consideration may be given to increasing the sample size from 360 evaluable patients at Week 24 (approximately 477 randomized patients) to 421 evaluable patients at Week 24 (approximately 540 randomized patients). For sample size estimation, evaluable patients are those who have observed data at Week 24 regardless of whether they are on treatment or not. As the sample size re-estimation will be based on pooled SD of the aggregate blinded data, no alpha adjustment will be required for this activity.

**Table 2: Sample Size Re-Calculation Depending on Common SD at Week 24**

| Common SD at Week 24 | >17-≤18 | >18-≤19 | >19-≤20 |
|---|---|---|---|
| Total Number of Randomized Patients | 540 | 594 | 666 |
| Total Number of Evaluable Patients Anticipated at Week 24 | 421 | 463 | 519 |

An independent Data Monitoring Committee (DMC) will periodically assess patient safety in an unblinded manner during the course of the study. No unblinded data will be accessible to site staff, the Sponsor, study monitors, and personnel of the electronic data capture (EDC) vendors before the database is locked. The specific activities and responsibilities of the DMC are defined in the DMC Charter for CY 4031. Based on the totality of available data, the DMC will make an overall recommendation to the Sponsor whether to continue the study according to the protocol or whether to make any changes in study conduct to protect the safety and welfare of the patients participating in the study. It has been requested that the DMC not make a recommendation that the trial be discontinued early for treatment benefit. Therefore, no alpha adjustment is required for the interim data review by DMC.

# 4. ANALYSIS SETS AND SUBGROUPS

This section describes the analysis sets that will be used in the planned and/or any potential ad hoc analyses and subgroup analyses.

## 4.1. Analysis Sets

### 4.1.1. Full Analysis Set (FAS)

The FAS will consist of all randomized patients who received at least one dose of study medication during the randomized Double-Blind, Placebo-Controlled and had at least one post-randomization efficacy assessment. The FAS will be analyzed as randomized.

### 4.1.2. Per Protocol Set (PPS)

The PPS will consist of all FAS patients who complete a minimum of 20 weeks of double-blind, placebo-controlled treatment (defined as reaching the Week 24 visit with no more than 4 cumulative weeks of treatment interruption), have at least one post-baseline efficacy assessment during treatment with double-blind study drug, and have no major protocol violations. The PPS will be analyzed as treated.

### 4.1.3. Safety Analysis Set (SAS)

The SAS will consist of all patients who receive any study medication, including patients in the Open-Label Phase. Safety analyses in each of the phases (i.e., Open-Label Phase, Double-Blind, Placebo-Controlled Phase, and Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase) will be based on a subset of patients in SAS who receive any study medication during the corresponding phase.

### 4.1.4. Pharmacokinetic Evaluable Data Set (PKEDS)

The PK Evaluable Data Set will consist of all randomized patients with at least one evaluable pharmacokinetic (PK) concentration, provided they have no major protocol violations that could affect the PK of *tirasemtiv* or riluzole.

### 4.1.5. All Screened Patients

The All Screened Patients will consist of all patients who signed informed consent and have been screened for this study. The All Screened Patients set is used to summarize reasons for screen failures.

## 4.2. Use of Analysis Sets in Different Analyses

The analysis datasets to be used are described in Table 3. Analyses will not be repeated on the PPS if the number of patients in the PPS $\geq$ 90% of the number of patients in the FAS.

**Table 3: CY 4031 Analysis Datasets**

| Analyses | FAS | PPS | SAS |
|---|---|---|---|
| Subject Disposition | | | √ |
| Time to Premature Discontinuation | | | √ |
| Demographics and Baseline Characteristics | √ | √ | √ |
| Medication History | | | √ |
| ALS History | | √ | √ |
| Medical History | | | √ |
| Efficacy | √ | √ | |
| Extent of Exposure | | | √ |
| Safety Measurements | | | √ |
| Concomitant Medications | | | √ |

## 4.3. Pooled Sites

Study site pooling will be performed based on the number of patients included in the FAS at each study site. Sites will be pooled by country and then by geographic proximity within country if there are more than 40 patients in one country. Sites will be pooled across countries if there are fewer than 40 patients in the FAS within a country. The plan for pooling of study sites is presented in Appendix B.

## 4.4. Subgroup Efficacy Analyses and Covariates

### 4.4.1. Subgroup Efficacy Analyses

The following subgroups are specified for the FAS:

1. Percent predicted SVC at baseline (<median, ≥median)
2. Body weight at baseline (<median, ≥median)
3. BMI at baseline (<median, ≥median)
4. Riluzole (use, non-use)
5. Randomized target dose group (placebo, *tirasemtiv* target dose group)
6. Maintenance dose group (See Section 4.4.1.1)
7. Sex
8. Age group (<65, ≥65 years old)
9. Race (White, Non-White)
10. Geographic region (North America, Europe)
11. Anatomic site of disease onset (bulbar, limb)

12. Time from ALS diagnosis (<median, ≥median; <1 year, ≥1 year)
13. ALSFRS-R at baseline (<median, ≥median)
14. Time from symptom onset (<median, ≥median)

Additional subgroups for exploratory analyses are as follows:

15. Score <4 on either ALSFRS-R items 10 or 11 at baseline (12 must be 4 at baseline) versus scores of 4 on both ALSFRS-R items 10 and 11
16. Patients with scores of 2 or more on all items of ALSFRS-R, SVC percent predicted of at least 80% at baseline, definite or probable ALS and time from symptom onset 2 years of less

#### 4.4.1.1. Maintenance Dose Group Definitions

Patients will be assigned to maintenance dose groups based on both their Week 8 visit prescribed dose and on their adjusted Week 8 visit dose through their Week 24 visit. Week 8 is designated as the end of the titration period.

Patients who discontinued study drug prior to or at the Week 8 visit will not be included in maintenance dose subset analyses of the primary endpoint, as they are considered to have discontinued study drug prior to establishment of a maintenance dose.

Patient subgroups based on maintenance dose will be determined for patients who continued to receive study drug after their Week 8 visit. The two methods of assigning patients to maintenance dose groups are as follows:

Week 8 Dose. Patients will be assigned to maintenance dose groups based on the dose prescribed at their Week 8 visit:

a) Placebo
b) 250 mg/day group
c) 375 mg/day group
d) 500 mg/day group

Adjusted Week 8 Dose through Week 24. Adjustments to the Week 8 visit prescribed dose will be made based on the average total dose received between Weeks 9 through 24:

Adjusted Week 8 Dose = Total Dose Weeks 9-24 / Maintenance Period Duration,

where,

Total Dose Weeks 9-24 = cumulative total dose from the Week 8 visit through last dose date on or prior to the Week 24 visit

Maintenance Period Duration = Week 24 visit date – Week 8 visit date + 1

Patients will be assigned to maintenance dose groups based on their Adjusted Week 8 Dose:

a) Placebo
b) 125 mg/day group (Dose < 187.5 mg/day)
c) 250 mg/day group (187.5 mg/day ≤ Dose < 312.5 mg/day)
d) 375 mg/day group (312.5 mg/day ≤ Dose < 437.5 mg/day)

e) 500 mg/day group (Dose $\geq$ 437.5 mg/day)

### 4.4.2. Covariates

If an imbalance in a baseline demographic characteristic is observed, each relevant factor and its interaction with treatment will be examined for the primary endpoint in the FAS.

Significant factors (with a p-value $\leq$0.05) will be included in the model for supportive analyses of the primary endpoint.

For secondary or tertiary endpoints related to skeletal muscle function (defined in Section 7.1.2 and Section 7.1.3), the interaction of treatment-by-riluzole use/non-use will also be included in the models.

# 5. HYPOTHESIS TESTING AND TREATMENT COMPARISONS

## 5.1. Hypothesis Testing

The primary analysis is to test the global null hypothesis that there is no treatment difference in the change from baseline in percent predicted SVC at Week 24 between patients in the FAS randomized to placebo and those randomized to *tirasemtiv* (pooled three target dose levels) during the first 24 weeks of double-blind, placebo-controlled treatment. This hypothesis will be tested at a 2-sided 5% significance level.

Null hypothesis tests for selected secondary endpoints will also be performed and the family-wise error rate for these hypotheses will be maintained at the 2-sided 5% significance level through a pre-specified order using a closed testing procedure detailed in Section 7.2.9 Multiplicity. Hypotheses other than those specified above (e.g. comparisons performed at different time points for example, Week 12 during the study) will be tested in an exploratory fashion at the 5% significance level with no adjustments made for multiple comparisons.

# 6. STUDY POPULATION

## 6.1. Patient Disposition

Patient disposition will be presented for the SAS. All patients who discontinue prematurely from the treatment and the study will be listed with age, sex, race, date and time of last dose, the corresponding study week, and the randomized target dose group and maintenance dose level established during the Double-Blind, Placebo-Controlled Phase if applicable, date of last visit, date of early termination, and reasons for discontinuation. Summary of patients' dispositions will be presented by randomized target dose group and overall for each phase separately as shown below.

**Open-Label Phase**

A table will present the number of patients:

- who were enrolled and received open-label treatment (i.e., the SAS)
- who completed the Open-Label Phase and were eligible to be randomized
- who did not tolerate open-label treatment and therefore were not eligible to be randomized to the Double-Blind, Placebo-Controlled Phase.

The reasons for patients not being eligible to be randomized will also be provided.

**Double-Blind, Placebo-Controlled Phase**

The number of patients who were randomized will be presented by randomized target dose group, treatment group (placebo and pooled *tirasemtiv* dose groups) and overall. The number of patients who were dosed with double-blind study drug will be presented by randomized target dose group, treatment and overall. The number of patients who completed double-blind dosing, and who discontinued study treatment early from the 48-week Double-Blind, Placebo-Controlled Phase will be presented by randomized target dose group, treatment and overall. Patients who discontinued the study early from the 48-week Double-Blind, Placebo-Controlled Phase will be summarized by those who agreed and those who did not agree to be followed. A summary will show reasons for early discontinuation of study treatment as recorded on the End of Study Treatment page of the electronic case report form (eCRF) and reasons for early discontinuation of study as recorded on the End of Study page of the eCRF.

Similar Summaries will be presented for patients who completed 24 weeks of the Double-Blind, Placebo-Controlled Phase and for patients who terminated early before completing 24 weeks of treatment with their reasons for early discontinuation from study treatment.

**Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase**

The number of patients randomized in the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase will also be presented by the randomized target dose group, placebo/placebo, *tirasemtiv*/placebo, *tirasemtiv*/*tirasemtiv* and overall. Among these patients, the number of patients who completed the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase and who discontinued early will be presented by randomized target dose level and overall. Reasons for early discontinuation from the study treatment from the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase as recorded on the End of Study Treatment page of the

eCRF will also be summarized. A summary will present reasons for early discontinuation from the study from the Double-Blind, Placebo-controlled, *Tirasemtiv* Withdrawal Phase as recorded on the End of Study page of the eCRF.

**Double-Blind, Placebo-Controlled Phase (i.e., 48 weeks Double-Blind, Placebo-Controlled Phase, Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase and Follow-Up Period)**

The denominator for this summary is the number of patients randomized in the Double-Blind, Placebo-Controlled Phase. The number of patients who completed the Double-Blind, Placebo-Controlled Phase including the *Tirasemtiv* Withdrawal Phase and follow-up period and the number of patients who did not complete the study will be presented by randomized target dose level and overall.

## 6.2. Demographics and Baseline Characteristics

Patient listings will be provided.

Patient demographics and other baseline characteristics will be summarized by randomized target dose group and overall in the FAS, PPS and SAS.

Demographic characteristics will include age, sex, race, ethnicity, height, weight, and BMI. Other baseline characteristics include baseline SVC (percent predicted and raw volume in liters), ALSFRS-R total score and ALSFRS-R score of the three respiratory subdomains of the ALSFRS-R (i.e., items 10, 11 and 12), SNIP, and baseline ALSAQ-5 and Epworth Sleepiness Scale. Demographic and baseline characteristics summaries will present the number of patients taking and not taking riluzole, time since diagnosis and time since symptom onset.

Patient disposition will be summarized for the SAS, the FAS and the PPS overall and by riluzole use/non-use. Descriptive statistics in terms of sample size, mean, median, SD and range will be presented for continuous variables. Frequency distributions in terms of number and percentage of patients will be presented for categorical variables.

Demographics and baseline characteristics will be examined for baseline imbalances for the FAS and the PPS between pooled active treatment dose levels and placebo. Categorical variables (e.g., sex) will be analyzed using Cochran-Mantel-Haenszel (CMH) tests; van Elteren tests will be used to analyze ordinal categorical measures stratified by riluzole use/non-use and pooled sites. Continuous variables (e.g., weight) will be analyzed using an analysis of variance (ANOVA) model with treatment group and riluzole use/non-use and pooled site as fixed effects.

## 6.3. Medical History

ALS history and diagnosis will be summarized for the SAS overall and by riluzole use/non-use. Variables will include ALS family history, site of onset, El Escorial criteria for ALS, and clinical region. General medical history data will be summarized for the SAS and for SAS patients who received at least one dose of double-blind study medication. The number and percentage of patients with each medical history item will be summarized by randomized target dose group and overall, and by system organ class and preferred term. Patient listings will be sorted by subject identification number and randomized target dose group and system organ class and preferred term.

## 6.4. Medication History and Concomitant Medication

Medical history and concomitant medication will be summarized for the SAS.

The analyses will describe medications other than the study medication taken during the study and reported on the eCRF. Medications with end dates that are 7 days or more prior to the first dose of study medication will be summarized as medication history. Medications with start dates that are 28 days after the last dose will be excluded from the concomitant medication summary. The World Health Organization (WHO) Drug dictionary will be used to classify concomitant medications by therapeutic class (ATC Class 3) and preferred name. If ATC Class 3 is not available, ATC Class 2 will be used. Coding will be performed using the "WHO DRUG ENHANCED+HERBAL format B2 June 2015" WHO Drug dictionary. The clinical study report will note the version of the WHO Drug dictionary.

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 concomitant medication.

Concomitant medication and medication history summaries will be presented for enrolled patients who failed the Open-Label Phase, as well as for patients who were randomized in the Double-Blind, Placebo-Controlled Phase by randomized target dose group and overall, and by WHO Drug ATC codes Level 3 and preferred name. The number and percentage of patients who received medications will be calculated. Multiple drug usage by a patient will be counted only once for each therapeutic class and preferred name.

In addition, a listing of all medications taken from screening through the end of study will be provided.

# 7. EFFICACY ANALYSES

## 7.1. Efficacy Endpoints

### 7.1.1. Primary Endpoint

The primary endpoint is the change from baseline to Week 24 of the Double-Blind, Placebo-Controlled Treatment Phase in percent predicted SVC.

### 7.1.2. Secondary Endpoints

The following secondary endpoints are listed in the sequence that they will be analyzed in a closed testing procedure if the primary efficacy analysis is significant as defined in Section 7.2.9:

1. The following two secondary endpoints will be tested simultaneously.
   - Change from baseline in the ALSFRS-R respiratory domain score (i.e., the sum of the scores of items 10, 11, and 12) to the end of 48 weeks of the double-blind, placebo-controlled treatment and
   - Slope of the mega-score of muscle strength during 48 weeks of the double-blind, placebo-controlled treatment
2. 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 use of non-invasive ventilation for ≥ 22 hours per day for ≥10 consecutive days) or death during all 48 weeks of double-blind, placebo-controlled treatment
3. Time to the first occurrence of a decline in SVC to ≤ 50% predicted or the onset of respiratory insufficiency or death during all 48 weeks of double-blind, placebo-controlled treatment
4. Change from baseline in the ALSFRS-R total score to the end of 48 weeks of the Double-Blind, Placebo-Controlled Phase
5. Time to the first use of mechanical ventilatory assistance or death during all 48 weeks of double-blind, placebo-controlled treatment. 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.

### 7.1.3. Tertiary Endpoints

Tertiary endpoints are presented grouped by their similarities to one another. The prospectively defined tertiary endpoints listed below will be the subject of descriptive, exploratory analyses as defined in this plan.

1. "Time to Event" endpoints including:
   a. Time to the first occurrence of a decline in SVC to ≤50% predicted or the onset of respiratory insufficiency or death during the first 24 weeks of double-blind, placebo-controlled treatment

b. Time to the first occurrence of a decline from baseline in percent predicted SVC ≥10 percentage points or the onset of respiratory insufficiency or death during the first 24 weeks of double-blind, placebo-controlled treatment
c. Time to the first occurrence of a decline from baseline in percent predicted SVC ≥20 percentage points or the onset of respiratory insufficiency or death during the first 24 weeks of double-blind, placebo-controlled treatment
d. Time to the first occurrence of a decline in the respiratory components of the ALSFRS-R (i.e., items 10, 11, and 12) or death during the first 24 weeks of double-blind, placebo-controlled treatment
e. Time to the first occurrence of a decline in either of the ALSFRS-R items 11 or 12 or death during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment
f. Time to the first occurrence of a decline in the ALSFRS-R item 12 or death during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment
g. Time to the first occurrence of the first use of mechanical ventilatory assistance or death during the first 24 weeks of double-blind, placebo-controlled treatment

In addition, each of the composite endpoints a, b and c listed above will be analyzed with "first use of mechanical ventilatory assistance" in place of "respiratory insufficiency" as below.

h. Time to the first occurrence of a decline in SVC to ≤50% predicted or first use of mechanical ventilatory assistance or death during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment
i. Time to the first occurrence of a decline from baseline in percent predicted SVC ≥10 percentage points or first use of mechanical ventilatory assistance or death during the first 24 weeks of double-blind, placebo-controlled treatment
j. Time to the first occurrence of a decline from baseline in percent predicted SVC ≥20 percentage points or first use of mechanical ventilatory assistance or death during the 48 weeks of double-blind, placebo-controlled treatment

Additional time to event endpoints of interest:

k. Time to the first occurrence of death or initiation of regular use of non-invasive ventilation during the first 24 weeks of double-blind, placebo-controlled treatment. 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 as collected on the patient outcome CRF page.
l. 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) or death during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment

2. "Responder analyses" including:

   a. Proportion of patients with no decline from baseline in percent predicted SVC, free from respiratory insufficiency and alive during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment

b. Proportion of patients with a decline from baseline ≤6 percentage points in percent predicted SVC, free from respiratory insufficiency and alive during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment
c. Proportion of patients with a decline from baseline ≤10 percentage points in percent predicted SVC, free from respiratory insufficiency and alive during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment
d. Proportion of patients with a decline from baseline ≤20 percentage points in percent predicted SVC, free from respiratory insufficiency and alive during the first 24 weeks and during all 48 weeks of double-blind, placebo-controlled treatment
e. Proportion of patients who do not meet the definition of non-self-sufficiency (NSS) at baseline and continue not to meet the definition as evaluated at Weeks 4, 12, 24, 36, and 48, when applicable. NSS is defined as a score of 2 or lower on at least one of the three ALSFRS-R items for swallowing, cutting food and handling utensils, or walking
f. Proportion of patients whose decline from baseline in ALSFRS-R score of the three respiratory domains of the ALSFRS-R (i.e., items 10, 11, and 12) is less than or equal to 2 points at the end of 48 weeks of double-blind, placebo-controlled treatment

3. Change from baseline to 24 weeks of double-blind, placebo-controlled treatment in the following measures:

   a. ALSFRS-R total score
   b. ALSFRS-R score of the three respiratory subdomains of the ALSFRS-R (i.e., items 10, 11, and 12)
   c. Muscle strength as determined by the mega-score of:

   - Elbow flexion (bilateral)
   - Wrist extension (bilateral)
   - Knee extension (bilateral)
   - Ankle dorsiflexion (bilateral)
   - Handgrip strength (bilateral)

   d. Sniff Nasal Inspiratory Pressure (SNIP)

4. Slopes of the changes from baseline in percent predicted SVC, ALSFRS-R, mega-score of muscle strength, and SNIP:

   a. From baseline to 24 weeks of the randomized, Double-Blind, Placebo-Controlled Phase
   b. From baseline to 48 weeks of the randomized, Double-Blind, Placebo-Controlled Phase (excluding muscle strength mega-score)
   c. From the end of 24 weeks of the randomized, Double-Blind, Placebo-Controlled Phase to the end of the Double-Blind, Placebo-Controlled Phase at 48 weeks

5. Changes in percent predicted SVC, muscle strength mega-score, and SNIP from baseline to the end of Week 48 of the Double-Blind, Placebo-Controlled Phase

6. Changes in percent predicted SVC, ALSFRS-R total score, muscle strength mega-score, and SNIP from baseline to the end of Week 12 of the Double-Blind, Placebo-Controlled Phase
7. Slope of the change from baseline in mega-score of muscle strength from baseline to 12 weeks of the Double-Blind, Placebo-Controlled Phase

#### 7.1.4. Additional Endpoints

1. ALSAQ-5 individual questions and total score change from baseline to the end of Week 24
2. Epworth Sleepiness Scale change from baseline to the end of Week 24
3. Caregiver burden change from baseline to the end of Week 24
4. ALSAQ-5 individual questions and total score change from baseline to the end of Week 48
5. Epworth Sleepiness Scale change from baseline to the end of Week 48
6. Caregiver burden change from baseline to the end of Week 48

## 7.2. Statistical Analyses of Treatment Effects

The effect of *tirasemtiv* compared to placebo will be presented as 1) the least squares (LS) mean differences for continuous repeated measures efficacy endpoints based on analysis of covariance (ANCOVA) repeated measures models, 2) the odds ratios estimated from the logistic regression models for the endpoints that are assessed by proportion and 3) hazard ratios estimated from the proportional hazard Cox regression models for time to event endpoints. For each variable, the LS means for *tirasemtiv* group (pooled three target dose levels based on sample size), each target dose level and the placebo group, the LS means for the difference between *tirasemtiv* and placebo and the associated 95% CIs and p-value will be provided.

Listings will present the efficacy measurements by patient identification number, randomized target dose group and treatment visit week.

All efficacy analyses will be performed in the FAS.

#### 7.2.1. Primary Analysis

The primary analysis is to test the global null hypothesis that there is no treatment difference in the change from baseline in percent predicted SVC at Week 24 between patients randomized to placebo and those randomized to *tirasemtiv* (pooled three target dose levels) during the double-blind, placebo-controlled treatment in the FAS.

The analysis will be performed using a repeated-measures mixed model with restricted maximum likelihood method (SAS® PROC MIXED default). The model will include terms for treatment, baseline, pooled site, visit, and riluzole use/non-use as well as interaction terms for treatment-by-visit and baseline-by-visit with an unstructured covariance matrix. If a 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 before database lock and unblinding for the final analysis of the primary endpoint. The treatment variable will be introduced to the mixed model with four categories (i.e., placebo and the three target dose levels). The difference in the primary endpoint of the overall *tirasemtiv* effect relative to placebo will be calculated in an estimate statement. See Section 13, Statistical Codes for details.

In order to address the missing data issue due to the early treatment terminations, this study will continue to follow patients and collect outcome data after patients discontinue the study medication. If there are more than 5% missing data at Week 24, the primary analysis will have multiple imputations of missing within each treatment arm before applying the above mentioned ANCOVA model.

### 7.2.2. Secondary and Tertiary Efficacy Analyses

All analyses will be performed with the methods below for all secondary and tertiary endpoints for the first 24 weeks of the Double Blind, Placebo-Controlled Phase and the 48 weeks of the Double Blind, Placebo-Controlled Phase separately. For endpoints related to skeletal muscle function, the models will include a term for interaction of treatment-by-riluzole.

1. Secondary or tertiary endpoints of change from baseline parameters will be analyzed using methods analogous to those used for the primary efficacy analysis. As for the primary analysis, the treatment effect variable will have four levels (placebo and three *tirasemtiv* target dose levels). Comparisons between each randomized target dose and placebo will be obtained from the LS means estimates. The overall *tirasemtiv* effect relative to placebo will be calculated using SAS's ESTIMATE statement.
2. For binary variables, the proportion of patients with an event will be provided. Odds ratios and their 95% confidence intervals (CI), as well as p-values, will be calculated using conditional logistic regression for the difference between *tirasemtiv* and placebo, stratified by pooled site with terms of baseline, treatment, and riluzole use/non-use in the model. Similar to the primary analysis model, the treatment effect variable will have four levels including placebo and the three target dose levels. Estimates for all *tirasemtiv* doses pooled will be obtained from SAS's ESTIMATE statement. An additional generalized estimating equation (GEE) proportional odds analysis will be conducted to evaluate the strength of association between the responding levels in change from baseline of % predicted SVC (0, > 0 - ≤ 6%, > 6 - ≤ 10%, > 10 - ≤ 20%, and > 20%) at the end of Week 24 treatment) and the randomized target dose group using a GENMOD ordinal model.
3. For time to event variables, a stratified proportional hazards Cox regression model will be used to estimate the hazards ratio and its 95% CI between active treatment group and placebo. The model will include treatment and covariates of baseline SVC value, and riluzole use/non-use, stratifying by pooled site. Similar to the primary analysis model, treatment effect variable will have four levels, placebo and the three target dose levels. Estimates for all *tirasemtiv* doses pooled will be obtained from an estimate statement. In an additional analysis, the NSS status at Week 4 or other applicable time points will also be added as a covariate to the above-mentioned Cox model to examine the prognostic effect on the probability of mechanical ventilatory assistance or death.

4. Each slope endpoint will be analyzed using a mixed model that will include treatment, the baseline value, pooled site, time, and riluzole use/non-use as well as interaction terms for treatment-by-baseline, and treatment-by-time. The model will assume a random slope effect. Slope endpoints will be compared between the patients randomized to all *tirasemtiv* target dose levels and those randomized to placebo during the Double-Blind, Placebo-Controlled Phase for the first 24 weeks and the 48 weeks of the Double-Blind Placebo-Controlled Phase separately. "Time" is the number of days from baseline assessment. The model will include the baseline time point with non-intercept specified. The slope estimate for each *tirasemtiv* target dose group will be obtained from the same model but the treatment effect variable will have four levels (placebo and three *tirasemtiv* target dose levels). Slopes of change from the end of 24 weeks to end of 48 weeks of the Double-Blind, Placebo-Controlled Phase will be plotted and examined using the same model with time as the number of days from the end of Week 24 and change from the end of 24 weeks as dependent variable.
5. Relationships between change from baseline in percent predicted SVC and change from baseline in ALSFRS-R respiratory domain score at Week 24 and Week 48 will be evaluated graphically and also by the correlation coefficient.

### 7.2.3. Rebound Effect Analyses

The potential for a rebound effect following withdrawal of *tirasemtiv* (i.e., acute worsening of function to a degree significantly worse than the decrement over time associated with placebo treatment) will be assessed by comparing the change from baseline to 49 weeks and from baseline to 52 weeks for all primary, secondary, and tertiary change from baseline endpoints on *tirasemtiv* (48 weeks of the Double-Blind, Placebo-Controlled Phase)/placebo (4 weeks of the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase) versus placebo (48 weeks of the Double-Blind, Placebo-Controlled Phase)/placebo (4 weeks of the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase) for the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase. A model analogous to the primary analysis model will be used to explore rebound with data up to Week 52. Treatment effect variables will have three levels (i.e., *tirasemtiv*/placebo, *tirasemtiv*/*tirasemtiv* and placebo/placebo). The 95% CIs will be calculated for the difference in LS means between the placebo/placebo group and the *tirasemtiv*/placebo group as well as *tirasemtiv*/*tirasemtiv* group with placebo/placebo group as reference.

### 7.2.4. Additional Efficacy Analyses

Additional efficacy analyses based on the change from baseline of additional efficacy endpoints will be analyzed using the same method as described for the primary efficacy analysis.

### 7.2.5. Supportive Analyses

As a key supportive analysis, the primary efficacy endpoint will be analyzed for the FAS using rank transformation repeated measures ANCOVA including the same terms included in the primary efficacy analysis. Ranks will be applied to all observed changes from baseline (regardless of visits or treatments) for continuous variables in the model. The rank based analysis will be conducted as a supportive analysis.

If rank-based analysis results are discrepant from the primary MMRM analysis, then two additional rank-based analyses that make use of within-visit rankings will be implemented. For the first analysis, the rank of a patient at the previous visit will be carried forward to the visit where the extreme value was observed. Extreme values in the pooled data at each visit are defined as observations outside of Tukey's outer fences (Tukey, 1977), i.e. observations that are less than the 25% quartile - 3 times of the inter quartile range or greater than 75% quartile + 3 times of the inter quartile range.

The second exploratory rank-based analysis will explore the influence of dropouts on the rank-based analysis. The percent predicted SVC change from baseline will be ranked at each assessment time point; ranks for any extreme values will be adjusted as described in the previous paragraph. Patients who drop out prior to Week 24 will have their last available rank carried forward to Week 24 as described in (O'Brien, Zhang, & Bailey, 2005).

As supportive analyses, the primary efficacy endpoint will be analyzed for the FAS using a repeated measures model which will include terms of treatment, baseline, visit, and riluzole use/non-use as well as interaction term of treatment by visit.

A supportive analysis for the primary efficacy endpoint for the PPS using the same method as the primary efficacy analysis will be performed. Supportive analysis will also be performed including terms of unbalanced baseline characteristics factor(s) if significant (p-value <0.05) in the model for the primary efficacy analysis for the FAS.

### 7.2.6. Sensitivity Analyses

Several sensitivity analyses will be conducted.

1. First, the primary efficacy analysis will be repeated using multiple imputations with the Markov chain Monte Carlo method to impute missing data points for the actual change from baseline in percent predicted SVC. 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. The sensitivity analysis for the base case will use the change from baseline of all observed and the aforementioned imputed data in the primary analysis model.
2. Two additional sensitivity analyses will be conducted using the invocations of such multiple imputations with 5% and 10% pessimism for the imputed missing values at each visit for the active treatment group.
3. When imputed data no longer have compatibility with parametric assumptions, ANCOVA based on ranks subsequent to imputations will be used. In this analysis, imputed values subsequent to death will be set to the worst rank.
4. The primary efficacy analysis will be repeated for all randomized patients in the first 24 weeks of the Double-Blind, Placebo-Controlled Phase.
5. For time to event endpoints, the following imputation of event onset is considered and analyzed as a sensitivity analysis. If a subject early terminates from the double-blind, placebo-controlled treatment and events of interest have not been observed by the time of termination then, the event time will be imputed according to the exponential distribution within the randomized treatment group (pooled *tirasemtiv* vs. placebo).

6. A time to multiple events sensitivity analysis will be performed for the following endpoint using the Wei, Lin and Weissfeld (WLW) model: Time t confirmed (two consecutive values) decline from baseline in percent predicted SVC ≥20 percentage points, time to use of non-invasive ventilation ≥22 hours for ≥10 consecutive days, tracheostomy or death.

### 7.2.7. Subgroup Efficacy Analyses

Analyses of the primary efficacy endpoint and secondary endpoints evaluating the change from baseline in ALSFRS-R total score at the end of Double-Blind, Placebo-Controlled Phase and the slope of the muscle strength mega-score during the 48 weeks of Double-Blind, Placebo-Controlled Phase will be conducted in the subgroups defined in Section 4.4.1. The primary model will be fit for each of the subgroup level in the FAS, excluding pooled site in the analyses. The models will remove riluzole use/non-use effect for subgroup analyses by riluzole use/non-use.

Similar to the subgroup analyses for the primary endpoint, the same model used for each of the secondary endpoints will be fit for each level of the subgroups where the imbalances occurred for the primary endpoint.

The treatment effect variable used in the subgroup analyses will only include four levels (i.e., placebo and the three randomized *tirasemtiv* dose levels) and the difference of the overall *tirasemtiv* effect relative to placebo will be calculated in an estimate statement. Model-based results will not be provided for a subgroup level with fewer than 10 patients in any of the treatment groups for continuous variables or at least three events for time to event analyses.

### 7.2.8. Exploratory Analyses

Exploratory analyses using a multivariate composite endpoint will be based on the repeated measures change from baseline in percent predicted SVC and the time to onset of respiratory insufficiency or death. The clinically relevant thresholds used in the composite endpoint are declines from baseline of 10, 20 and 30 percentage points in percent predicted SVC. The first three events using the threshold is time to decline from baseline of 10, 20 and 30 percentage points in percent predicted SVC or onset of respiratory insufficiency or death. The final threshold endpoint is the time to onset of respiratory insufficiency or death. Patients are censored if patients do not meet any of the criteria set for each time to event during the time interval where the time to event is evaluated. For example, if a patient has experienced a decline in SVC of 10 percentage points at Week 8 but does not experience a decline of ≥20 percentage points from baseline and is free from respiratory insufficiency and alive through the end of Week 24, the patient's time to 1$^{st}$ event will be 8 weeks but will be censored at 24 weeks for the 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ time to event. The multiple time to events will be analyzed using approaches in (Saville, Herring, & Koch, 2010), WLW model with equal weights. A second composite endpoint based on the repeated measures of change from baseline in percent predicted SVC and the time to onset of first use of mechanical ventilatory assistance or death will be analyzed using an analogous method.

### 7.2.9. Multiplicity

The null hypothesis for the primary and secondary efficacy endpoints will be tested in a pre-specified order using a closed testing procedure. This procedure will maintain the family-wise error rate at two-sided significance level of 0.05 for all hypotheses tested in a confirmatory sense.

Step 1. The null hypothesis that there is no treatment difference in the primary efficacy endpoint at the end of the 24 weeks of double-blind, placebo-controlled treatment in the FAS between pooled three *tirasemtiv* target dose levels and placebo will be tested at the two-sided significance level of 0.05. If this hypothesis is rejected, testing will proceed to Step 2; otherwise testing will stop.

Step 2. The null hypothesis that there is no treatment difference in the secondary efficacy endpoint of change from baseline in ALSFRS-R respiratory domain score of the ALSFRS-R (i.e., the sum of scores for items 10, 11, and 12) at the end of 48 weeks of double-blind, placebo-controlled treatment in the FAS between pooled three *tirasemtiv* target dose levels and placebo ($H_{021}$) and the null hypothesis that there is no treatment difference in the secondary efficacy endpoint of the slope in muscle-strength mega-score during the 48 weeks of double-blind, placebo-controlled treatment in the FAS between pooled three *tirasemtiv* target dose levels and placebo ($H_{022}$) will be tested as follows using the Benjamini-Hochberg method described in (Somerville, Wilson, & Koch, 2005). The weighted significance level for $H_{021}$ and $H_{022}$ are 0.01 and 0.04 respectively.

i) Test both $H_{021}$ and $H_{022}$ at a two-sided significance level of 0.05. If both hypotheses are rejected, then the subsequent null hypothesis in Step 3 will be tested at a two-sided significance level of 0.05.

ii) If $H_{021}$ is not rejected at the two-sided 0.05 significance level, then the hypothesis $H_{022}$ will only be rejected if the p-value is less than the predetermined two-sided significance level of 0.04. Tests of further hypotheses in the hierarchy will not be carried out.

iii) If $H_{022}$ is not rejected at the two-sided 0.05 significance level, then the hypothesis $H_{021}$ will only be rejected if the p-value is less than the predetermined significance level of 0.01. Tests of further hypotheses in the hierarchy will not be carried out.

iv) If both $H_{021}$ and $H_{022}$ are not rejected at the two-sided significance level of 0.05, tests of further hypotheses in the hierarchy will not be carried out.

Step 3. The null hypothesis that there is no treatment difference in the secondary efficacy endpoint of time to the first occurrence of a decline from baseline in percent predicted SVC $\geq$20 percentage points or the onset of respiratory insufficiency or death at the end of the 48 weeks of double-blind, placebo-controlled treatment will be tested in the FAS between pooled three *tirasemtiv* target dose levels and placebo at the two-sided significance level of 0.05. If this hypothesis is rejected, testing will proceed to Step 4; otherwise testing will stop.

Step 4. The null hypothesis that there is no treatment difference in the secondary efficacy endpoint of time to the first occurrence of a decline in SVC to ≤50% predicted or the onset of respiratory insufficiency or death at the end of the 48 weeks of double-blind, placebo-controlled treatment in the FAS between pooled three *tirasemtiv* target dose levels and placebo will be tested at the two-sided significance level of 0.05. If this hypothesis is rejected, testing will proceed to Step 5; otherwise testing will stop.

Step 5. The null hypothesis that there is no treatment difference in the secondary efficacy endpoint of change from baseline in ALSFRS-R total score to the end of 48 weeks of double-blind, placebo-controlled treatment will be tested in the FAS between pooled three *tirasemtiv* target dose levels and placebo at the two-sided significance level of 0.05. If this hypothesis is rejected, testing will proceed to Step 6; otherwise testing will stop.

Step 6. The null hypothesis that there is no treatment difference in the secondary efficacy endpoint of time to the first use of assisted ventilation or death during all 48 weeks of double-blind, placebo-controlled treatment (defined as invasive or non-invasive ventilation for at least 2 hours over a 24 hour period for at least 5 consecutive days) will be tested in the FAS between pooled three *tirasemtiv* target dose levels and placebo at the two-sided significance level of 0.05.

# 8. SAFETY ANALYSES

Safety analyses will be based on the SAS overall and by the phases of the study (i.e., Open-Label Phase; all 48 weeks of the Double Blind, Placebo-Controlled Phase; the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase; and until the end of follow-up period) as well as subgroup analyses by riluzole use/non-use.

Safety parameters include AEs, concomitant medications (as described in Section 6.4), clinical laboratory evaluations, vital signs, ECGs, fall assessments, neurological examinations, and physical examinations.

## 8.1. Evaluation of Extent of Treatment Exposure

The extent of treatment exposure will be summarized for patients for the SAS overall and by phases of the study (i.e., Open-Label Phase, 48 weeks of the Double-Blind, Placebo-Controlled Phase and the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase). The summary will be presented by randomized target dose group for each phase.

The extent of treatment exposure summary for the 48 weeks of double-blind, placebo-controlled treatment will also be provided by the maintenance dose (Section 4.4.1.1) established during the Double-Blind, Placebo-Controlled Phase. A detailed patient listing with each study medication by date and time, dose amount, and reason full dose not taken will be provided.

## 8.2. Adverse Events

### 8.2.1. Treatment Emergent Adverse Events (TEAEs)

The Medical Dictionary for Regulatory Activities (MedDRA) will be used to classify AEs by system organ class and preferred term. The severity of AEs will be evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 4.0. Coding will be performed using version 18 of the MedDRA coding dictionary. As dictionary versions are constantly updated, use of the most recent version of MedDRA for the study will be evaluated prior to database lock.

#### 8.2.1.1. Definition of TEAEs in the Open-Label Phase

TEAEs in the Open-Label Phase are AEs that meet any of the following criteria:

1. An AE which was not present prior to the first dose of the open-label phase study medication with an onset before the first dose of the double-blind treatment
2. An AE which was present prior to the first dose of the open-label phase study medication but worsened in severity during open-label treatment and before the first dose of the double-blind treatment
3. An AE which was not present prior to the first dose of the open-label phase with an onset no later than 28 days after the last dose of the open-label phase study medication for subjects who did not receive any randomized study medication during the Double-Blind, Placebo-Controlled Phase.

#### 8.2.1.2. Definition of TEAEs in the Double-Blind, Placebo-Controlled Phase of the Study

The assignment of TEAEs to the randomized treatment during the 48 weeks of the Double-Blind, Placebo-Controlled Phase is complicated by the occurrence of TEAEs in the Open-Label Phase that continue without interruption into the 48 weeks of the Double-Blind, Placebo-Controlled Phase.

Assigning such TEAEs to the randomized treatment during the Double-Blind, Placebo-Controlled Phase may appear to be conservative; however, this "prevalence" approach will result in assigning to double-blind placebo those TEAEs that begin during open-label *tirasemtiv* and persist into the Double-Blind, Placebo-Controlled Phase after randomization to placebo but resolve shortly thereafter, thus potentially inflating inappropriately the reported incidence of TEAEs on placebo.

An "exclusionary" approach (which appeared to perform successfully in assigning such TEAEs to randomized treatment in the safety analyses of the Phase 2b study of *tirasemtiv* in ALS, CY 4026), is to assign such TEAEs to the randomized treatment during the Double-Blind, Placebo-Controlled Phase only if they persist beyond 96 hours or 4 days after the first dose of double-blind study medication. A potential limitation of this approach is that it, too, may potentially inflate inappropriately the reported incidence of TEAEs on placebo during the Double-Blind, Placebo-Controlled Phase for patients randomized to placebo, although likely to a lesser extent than the "prevalence" approach described above because of the number of TEAEs that will resolve during the first 96 hours of the Double-Blind, Placebo-Controlled Phase.

Finally, an alternative "incidence" approach is to assign such TEAEs to the randomized treatment during the Double-Blind, Placebo-Controlled Phase only if they worsen after the first dose of double-blind study drug. A potential limitation of this approach is that TEAEs that begin during open-label *tirasemtiv* and persist into the Double-Blind, Placebo-Controlled Phase after randomization to *tirasemtiv* (possibly for as long as the entire Double-Blind, Placebo-Controlled Phase) but never worsen would not be assigned to *tirasemtiv*, thus potentially diminishing inappropriately the reported incidence of TEAEs on *tirasemtiv*.

Summarizing TEAEs based on each of the three approaches for assigning AEs that persist from the Open-Label Phase into the Double-Blind, Placebo-Controlled Phase (i.e., "exclusionary," "prevalence," and "incidence" approaches) to the patients' randomized target dose group should help to assess the AE profile of *tirasemtiv* relative to placebo.

##### 8.2.1.2.1. TEAEs in the 48 weeks Double-Blind, Placebo-Controlled Phase

###### 8.2.1.2.1.1. Exclusionary Assignment

1. An AE which was not present before the first dose of the Open-Label Phase and began after the first dose of the double-blind, placebo-controlled phase study medication, or
2. An AE which was present before the first dose of the Open-Label Phase and increased in severity only after taking double-blind, placebo-controlled phase study medication during the Double-Blind, Placebo-Controlled Phase, or
3. A TEAE in the Open-Label Phase according to either of the first two definitions above in Section 8.2.1.1 which continues without interruption beyond 96 hours or 4 days into the

Double-Blind, Placebo-Controlled Phase; in these cases, AE onset will be the date of the first double-blind, placebo-controlled phase study medication

4. For patients who are not randomized into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase, the onset date is no later than 28 days after taking the last dose of study medication during the Double-Blind, Placebo-Controlled Phase. For patients who are randomized into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase, the onset date must be on or before the last dose of study medication in the Double-Blind, Placebo-Controlled Phase.

#### 8.2.1.2.1.2. Prevalence Assignment

1. An AE which was not present before the first dose of the Open-Label Phase and began after the first dose of the double-blind, placebo-controlled phase study medication, or
2. An AE which was present before the first dose of the Open-Label Phase and increased in severity only after taking double-blind study medication during the Double-Blind, Placebo-Controlled Phase, or
3. A TEAE in the Open-Label Phase according to either of the first two definitions above in Section 8.2.1.1 which continues without interruption into the Double-Blind, Placebo-Controlled Phase (in these cases, AE onset will be the date of the first double-blind, placebo-controlled phase study medication).
4. For patients who are not randomized into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase, the onset date is no later than 28 days after taking the last dose of study medication during the Double-Blind, Placebo-Controlled Phase. For patients who are randomized into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase, the onset date must be on or before the last dose of study medication in the Double-Blind, Placebo-Controlled Phase.

#### 8.2.1.2.1.3. Incidence Assignment

1. An AE which was not present before the first dose of the Open-Label Phase and began after the first dose of the double-blind, placebo-controlled phase study medication, or
2. An AE which was present before the first dose of the open-label phase and increased in severity only after taking double-blind, placebo-controlled study medication during the Double-Blind, Placebo-Controlled Phase, or
3. A TEAE in the Open-Label Phase according to either of the first two definitions above in Section 8.2.1.1 which continues without interruption into the Double-Blind, Placebo-Controlled Phase and worsens in severity during treatment in the Double-Blind, Placebo-Controlled Phase (in these cases, AE onset will be the date of worsening in severity).
4. For patients who are not randomized into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase, the onset date is no later than 28 days after taking the last dose of study medication during the Double-Blind, Placebo-Controlled Phase. For patients who are randomized into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase, the onset date must be on or before the last dose of study medication in the Double-Blind, Placebo-Controlled Phase.

#### 8.2.1.2.2. Definition of TEAEs in the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase

The assignment of TEAEs to treatment during the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase is complicated by the occurrence of TEAEs in the Double-Blind, Placebo-Controlled Phase that continue without interruption into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase. Consequently, similar to what was done to assign TEAEs that began during the Open-Label Phase to randomized treatment during the Double-Blind¸ Placebo-Controlled Phase, three methods of assignment will be employed in summarizing TEAEs during the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase, i.e., exclusionary, prevalence, and incidence.

##### 8.2.1.2.2.1. Exclusionary Assignment

1. An AE that began after the first dose of the Double-Blind¸ Placebo-Controlled *Tirasemtiv* Withdrawal Phase study medication, or
2. A TEAE in the Double-Blind, Placebo-Controlled Phase according to the Exclusionary Assignment described above in Section 8.2.1.2.1.1 which continues without interruption beyond 96 hours or 4 days into the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase (in these cases, AE onset will be the date of the first double-blind study medication for the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase).
3. The onset date is no later than 28 days after taking the last dose of study medication during the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase.

##### 8.2.1.2.2.2. Prevalence Assignment

1. An AE that began after the first dose of the Double-Blind¸ Placebo-Controlled *Tirasemtiv* Withdrawal Phase study medication, or
2. A TEAE in the Double-Blind, Placebo-Controlled Phase according to the Prevalence Assignment described above in Section 8.2.1.2.1.2 which continues without interruption into the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase (in these cases, AE onset will be the date of the first double-blind, placebo-controlled, *tirasemtiv* withdrawal phase study medication).
3. The onset date is no later than 28 days after taking the last dose of study medication during the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase.

##### 8.2.1.2.2.3. Incidence Assignment

1. An AE that began after the first dose of the Double-Blind¸ Placebo-Controlled *Tirasemtiv* Withdrawal Phase study medication, or
2. A TEAE in the Double-Blind¸ Placebo-Controlled Phase according to Incidence Assignment described above in Section 8.2.1.2.1.3 which continues without interruption into the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase and worsens in severity during treatment in the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase (in these cases, AE onset will be the date of worsening in severity).

3. The onset date is no later than 28 days after taking the last dose of study medication during the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase.

### 8.2.2. Summary of Treatment Emergent Adverse Events

TEAEs are summarized for the SAS. For the summaries by NCI-CTCAE grade, if more than one event occurs with the same preferred term per patient, the patient will be counted only once for that preferred term using the highest grade for the randomized target dose group or dose level.

**TEAEs During Open-Label Phase**

The denominator for these TEAEs are patients in the SAS who received at least one dose of study medication in the open-label phase. The number and percentage of patients with TEAEs in the open-label phase will be summarized by system organ class, preferred term and by whether patients were intolerant of open-label study medication (lead-in failure), or randomized to placebo or one of the three randomized doses of *tirasemtiv* and took at least one dose of double-blind study medication) and overall. The following adverse event summaries will be provided:

- TEAEs in open-label phase
- TEAEs leading to early termination in open-label phase
- Serious TEAEs
- TEAEs by NCI-CTCAE code
- TEAEs by relationship to the study medication
- TEAEs by riluzole use/non-use

**TEAEs During the 48 week Double-Blind, Placebo-Controlled Phase**

Patients who received at least one dose of study medication during the Double-Blind, Placebo-Controlled Phase will be included in TEAEs summaries for this period. The number and percentage of patients with TEAEs will be summarized by system organ class, preferred term, randomized target dose group and overall. TEAEs will also be summarized by system organ class, preferred term, dose level and overall.

The dose level for each TEAE is assigned as:

a. The total daily dose the patient received on the day prior to the TEAE onset date or the day prior to the date the TEAE worsened or
b. The last non-missing total daily dose if there are study medication interruptions when the TEAEs occur or
c. The last total daily dose if the TEAEs occur after the last dose or
d. For the "prevalence" and the "exclusionary" approaches, the dose level for TEAEs that start in the Open-Label Phase and continue without interruption (for "prevalence") or for ≥96 hours or 4 days (for "exclusionary") into the Double-Blind, Placebo-Controlled Phase will be assigned to placebo if the patient is randomized to placebo group or to the 250 mg/day group if the patient is randomized to one of the *tirasemtiv* dose groups.

The following AE summaries will be provided for each of the TEAE definitions (Prevalence, Exclusionary, and Incidence):

- TEAEs
- TEAEs leading to early termination in the Double-Blind, Placebo-Controlled Phase
- Serious TEAEs

In addition, the following AE summaries will be provided for the Exclusionary and Incidence TEAE definitions:

- TEAEs by NCI-CTCAE grade
- TEAEs by relationship to the study medication
- TEAEs by riluzole use/non-use

**TEAEs During the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase**

The summary will include only patients who were randomized and took at least one dose of study medication in the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase. TEAEs in the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase will be summarized by randomized target dose group in the *tirasemtiv* withdrawal phase (i.e., *tirasemtiv*/placebo [treatment assigned in the 48 week Double-Blind, Placebo-Controlled Phase]/ treatment assigned in the *tirasemtiv*-withdrawal phase), *tirasemtiv*/*tirasemtiv* or placebo/placebo. TEAEs in the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase will also be summarized by the dose level.

The dose level for each TEAE is assigned as:

a. The total daily dose the patient received on the day prior to the TEAE onset date or the day prior to the date the TEAE worsened or
b. The last non-missing total daily dose if there are study medication interruptions when the TEAEs occur or
c. The last total daily dose if the TEAEs occur after the last dose or
d. For the "prevalence" and the "exclusionary" approaches, the dose level for TEAEs in the 48 weeks of the Double-Blind, Placebo-Controlled Phase that continue without interruption (for "prevalence") or for ≥96 hours or 4 days (for "exclusionary") into the Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase will be assigned to placebo if the patient is randomized ($2^{nd}$ randomization) to the placebo group or to the dose level assigned in the Double-Blind, Placebo-Controlled Phase if the patient is randomized to one of the *tirasemtiv* dose groups.

The following summaries will be provided for each of the TEAE definitions (Prevalence, Exclusionary and Incidence).

- TEAEs
- TEAEs that led to early termination from the *tirasemtiv* withdrawal phase
- Serious TEAEs

In addition, the following AE summaries will be provided for the Exclusionary and Incidence TEAE definitions:

- TEAEs by NCI-CTCAE grade

- TEAEs by relationship to the study medication
- TEAEs by riluzole use/non-use

**TEAEs During the Double-Blind Phases**

The Double-Blind Phases include the following: Double-Blind, Placebo-Controlled Phase, Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase and the Follow-up Period). For patients in the SAS who took at least one dose during the Double-Blind, Placebo-Controlled Phase, TEAEs will be summarized by system organ class, preferred term, the randomized target dose group and overall. The following summaries will be provided for each of the Exclusionary and Incidence TEAE definitions:

- TEAEs
- TEAEs that led to early termination
- Serious TEAEs
- TEAEs by relationship to the study medication
- TEAEs by NCI-CTCAE grade
- TEAEs by riluzole use/non-use

**TEAEs During the Study**

**(Open-Label Phase, Double-Blind, Placebo-Controlled Phase, Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase and Follow-Up Period)**

For patients in the SAS who took at least one dose of study drug, TEAEs (Incidence) will be summarized by system organ class, preferred term, randomized target dose group (patients who were not randomized will be summarized under column 'Open-Label Lead-in failure') and overall. The following summaries will be provided.

- TEAEs
- TEAEs that led to early termination
- serious TEAEs
- TEAEs by relationship to the study medication
- TEAEs by NCI-CTCAE grade
- TEAEs by riluzole use/non-use

All AEs reported on the eCRF AE form will be included in the listing.

### 8.2.3. Imputation of Onset and Duration of TEAEs with Partial or Missing Dates

For TEAEs with incomplete date information recorded in the eCRF, the derivation of TEAE onset date, onset time, and duration will follow the following algorithms:

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, the month of AE onset is the same as the month of the first dosing date and the AE onset is after the first dosing date, then the first dosing date will be the imputed AE onset date.
- If no AE onset information is available, then the first dosing date will be the imputed AE onset date.

2. For missing AE end dates and times, the following algorithm will be used to derive the AE duration:
   - 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
     – For patients who discontinued early from double-blind, placebo-controlled treatment, the imputed AE end date will be on the later of the last dosing date + 28 days or the last visit or contact date.
     – For patients who completed the study, the imputed AE end date and time will be the date of the Follow-Up Visit.

Duration of AEs will be presented in days in the AE listing.

### 8.2.4. Time to First Onset and Duration of Dizziness

Time to onset of dizziness in the Open-Label Phase is calculated from the first dose of open-label *tirasemtiv* dose to onset of dizziness during the Open-Label Phase.

Time to onset of dizziness in the Double-Blind, Placebo-Controlled Phase is calculated as follows.

1. For patients not reporting dizziness at the time of their first dose of double-blind, placebo-controlled study medication, time to onset of treatment-emergent dizziness in the Double-Blind, Placebo-Controlled phase is calculated from the first dose of double-blind, placebo-controlled study medication to dizziness onset during the Double-Blind, Placebo-Controlled Phase.
2. For patients reporting dizziness at the time of their first dose of double-blind study medication, time to onset of treatment-emergent dizziness in the Double-Blind, Placebo-Controlled Phase will be calculated in each of the following ways:
   - Time to onset of dizziness is the time from the first dose of double-blind study medication to the time dizziness worsened during the Double-Blind, Placebo-Controlled Phase

- If the dizziness at the time of the first dose of the double-blind study medication resolves with 96 hours or 4 days, time to onset of dizziness is the time from the first dose of double-blind, placebo-controlled study medication to the time of the first recurrence of dizziness during the Double-Blind, Placebo-Controlled Phase
- If the dizziness at the time of the first dose of the double-blind study medication persists beyond 96 hours or 4 days after the first dose of double-blind study medication, time to onset of dizziness will be considered to be 96 hours after the first dose of double-blind, placebo-controlled study medication

The time to the first onset of dizziness will be summarized descriptively by randomized target dose group and overall in terms of median, 95% CIs of the median, Q1, Q3, and range, using the Kaplan-Meier method. Descriptive statistics for the duration of the first occurrence of dizziness will be summarized by randomized target dose group and overall in terms of number of patients with dizziness, median, 95% CIs of the median, Q1, Q3, and range. Summary of time to first onset of dizziness by riluzole use/non-use will also be provided.

Kaplan-Meier survival curves for time to the first onset of dizziness during the open-label phase, time to the first onset of dizziness during the 48 weeks of the Double-Blind, Placebo-Controlled Phase, and time to the second onset of dizziness during the 48 weeks of the Double-Blind, Placebo-Controlled Phase will be presented by randomized target dose group.

Patients without dizziness events will be censored at the end of each phase of the study.

Associations between the *tirasemtiv* plasma concentrations on Day 1 and Week 4/Early Termination prior to Week 4 (ET) and the presence of dizziness on the days of the Day 1 and Week 4/ET blood draws will be explored. The Day 1 percent of patients with dizziness will be summarized by *tirasemtiv* plasma concentration quartiles, where the quartiles are determined by the Week 4/ET concentration levels. The Week 4/ET percent of patients with dizziness will be summarized for the placebo group and by *tirasemtiv* plasma concentration quartiles.

## 8.3. Clinical Laboratory Evaluations

Clinical laboratory evaluations, including hematology, serum chemistry, and urinalysis, will be collected at Screening, Week -2, Week 4, Week 8, Week 24, Week 48, Week 52 and Week 56 (Follow-up Visit). Summary of clinical laboratory parameters are based on the SAS.

Descriptive statistics for observed values and changes from baseline will be summarized for each scheduled assessment visit week, by randomized target dose group, dose level and overall. Dose level is the total daily dose patients received the day prior to the clinical laboratory evaluation assessment date. Summaries of the last available on-drug assessments and at the follow-up visit also will be provided.

Baseline is defined as the last non-missing assessment, including unscheduled assessments, made prior to the initial dose of open-label study medication.

The number and percentage of patients with abnormal laboratory values for each study phase will be presented by randomized target dose group and dose level, by abnormal high or low and overall. The lower limit of normal (LLN) and upper limit of normal (ULN) provided by the laboratory will be the criteria used to determine abnormal laboratory values.

Severe potentially clinically significant (PCS) abnormal laboratory values are defined as Grade 3 or more laboratory abnormalities according to NCI-CTCAE Version 4.0. Summaries of the number and percentage of patients with severe PCS for each study phase will be provided by randomized target dose group, dose level and by abnormal high or low and overall.

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 indicated treatment and dose level, and overall.

A shift table for randomized and dosed patients will be provided for each study phase in the form of shifts from baseline NCI-CTCAE grade to the maximum grade post randomization by randomized target dose group, laboratory tests and overall. 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.

Clinical laboratory (hematology, serum chemistry, urinalysis, and other) values will be listed chronologically by patient, visit week, and the actual assessment date and time. Values outside the Laboratory's normal ranges will be flagged. Unscheduled laboratory values also will be included. A separate listing of patients reporting at one or more post-randomization CTCAE toxicity grade ≥3 laboratory test results will be provided.

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

## 8.4. Vital Signs

Vital signs, including blood pressure, pulse, respiration rate and weight were obtained at each clinic visit starting from the Screening Visit through the Follow-up Visit.

Descriptive statistics for absolute values and changes from baseline in vital sign parameters will be summarized for each visit, by randomized target dose group and dose level, overall and by phase. Dose level is the total daily dose patients received the day prior to the vital signs assessment date. Summaries of the last available on-drug assessments and at the follow-up visit also will be provided.

Baseline is defined as the last non-missing assessment, including unscheduled assessments, made prior to the initial dose of open-label study medication.

The number and percentage of patients with PCS abnormal vital signs will be presented by randomized target dose group, overall and by phase using the criteria specified in Table 4. In calculating percentages for each parameter, the denominator is the number of dosed patients with a non-PCS abnormal or missing baseline assessment and with at least one post-randomization assessment (patients with a PCS abnormal baseline vital sign value will be excluded from the denominator for that parameter); the numerator is the number of dosed patients with a non-PCS or missing baseline assessment and at least one PCS abnormal post-baseline assessment (including unscheduled assessments, and assessments at the Follow-Up Visit).

**Table 4: Criteria for Potentially Clinically Significant Vital Signs**

| Vital Sign Parameter | Flag | 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 bpm |
| | Low | <8 bpm |
| Pulse (bpm) | High | ≥120 bpm |
| | Low | ≤50 bpm |
| Weight (kg) | Clinically Significant | ≥5% reduction from baseline |

Shift tables for randomized and dosed patients will be provided for each study phase in the form of shifts from baseline PCS classification (normal, abnormal high and abnormal low) to the post randomization PCS abnormal high and post randomization PCS abnormal low separately by randomized target dose group, vital sign parameters (except weight) and overall. PCS post randomization classification is based on the maximum post randomization values and the minimum post randomization values. Patient will have both classifications of the post randomization maximum and minimum values if both meet the PCS criteria. Shifts for the last available on-drug assessments and at the follow-up visit also will be included.

Vital signs will be listed chronologically by patient, visit, and the actual assessment date and time. PCS values will be flagged and unscheduled vital sign values also will be noted.

The change from baseline in weight at each study visit will be explored in the SAS in the Double-Blind, Placebo-Controlled Phase. The model will be analogous to the one used for the primary endpoint.

The association between the concentration of *tirasemtiv* and the change from baseline in vital signs might be explored.

## 8.5. Electrocardiogram

A 12-lead electrocardiogram (ECG) will be obtained at Screening and at Week 8, Week 24, Week 48, Week 52 and Follow-up Visit.

Descriptive statistics for observed values and changes from baseline in ECG intervals will be summarized for each visit, by randomized target dose group, dose level, overall and by phase. Baseline is defined as the last non-missing assessment, including unscheduled assessments, made prior to the initial dose of open-label study medication. Summaries of the last available on-drug assessments and at the follow-up visit also will be provided. Dose level is the total daily dose patients received the day prior to the ECG assessment date.

The number and percentage of patients with abnormal high and PCS abnormal high ECG intervals (except uncorrected QT interval) will be presented by randomized target dose group, dose level, overall and by phase, using the criteria specified in Table 5.

In calculating percentages of patients with abnormal high 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 high 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).

Shifts in ECG interpretations from baseline to each post-baseline assessment will be provided. The categories are Normal, non-PCS Abnormal, Abnormal-PCS, Not Evaluable, and Total.

12-lead ECG parameters (PR interval, QRS interval, QT interval, and QTc interval [using both Bazett's and Fridericia's corrections for heart rate]), as well as ECG interpretations, will be listed by patient, visit, and the actual assessment date and time. PCS ECG intervals as described below in Table 5 will be flagged. Unscheduled ECG parameters also will be flagged in the listing.

**Table 5: Criteria for Abnormal High and PCS High ECG Values**

| ECG Variable | Units | Upper Limit of Normal | PCS High Values |
|---|---|---|---|
| QRS Interval | msec | 80 | ≥120 |
| PR Interval | msec | 200 | ≥240 |
| QTcB Interval | msec | Males: 450<br>Females: 460 | >500 |
| QTcF Interval | msec | Males: 450<br>Females: 460 | >500 |

Bazett's QTc interval is derived as: QTcB=QT Interval (msec) / (RR (msec)/1000)**(1/2);
Fridericia's QTc interval is derived as: QTcF=QT Interval (msec) / (RR (msec)/1000)**(1/3);

The association between concentration of *tirasemtiv* and the change from baseline in ECG parameters might be explored.

## 8.6. Falls Assessment

A falls assessment will be performed at each clinic visit, starting at Week -2 and through the Follow-Up Visit.

The number and percentage of patients with falls will be presented at baseline (i.e., from Screening to the first dose of open-label study medication), and after baseline, by randomized target dose group, dose level, overall and by phase. Dose level is the total daily dose patients received the day prior to the date of fall. Patients' activities at the time of the falls, physical symptoms immediately preceding the falls, and injuries resulting from the falls will be tabulated by randomized target dose group, dose level, overall and by phase. Falls are not recorded as AEs;

however, physical symptoms immediately preceding the falls are recorded as AEs if they are new or represent a clinically significant worsening of a symptom present at baseline.

## 8.7. Neurological Examinations and Ashworth Score

Neurological examinations will be obtained at Screening Visit, at Week 24, Week 48 and at the Follow-up Visit.

Shift in neurological examination results from Screening to each post-baseline assessment by visit, randomized target dose group, dose level, overall and by phase will be provided for each test, using the categories described on CRF. Dose level is the total daily dose patients received the day prior to the neurological examination assessment date.

For Ashworth tests, the average score of the four joints will be summarized descriptively by visit, randomized target dose group, dose level, overall and by phase.

Individual neurological examination results will be listed for each test chronologically by patient, visit, and the actual assessment date and time.

## 8.8. Suicidality Assessment

The number and percentage of patients expressing suicidal ideation and its intensity will be presented by randomized target dose group, dose level, overall and by phase. Dose level is the total daily dose patients received the day prior to the suicidality assessment date.

Individual suicidal ideation results will be listed by patient, visit, and the actual assessment date and time.

## 8.9. Other safety assessments

The incidence density of death will be provided by randomized target dose group, adjusting for each patient's actual treatment duration. The ratio of the incidence density of death and 95% CIs for *tirasemtiv* relative to placebo will be calculated using a Poisson regression. The p-value from the exact Poisson test comparing the mortality rates for each active to placebo will be presented. The density of other rare events of interest will also be analyzed similarly.

# 9. PHARMACOKINETIC AND PHARMACOKINETIC/PHARMACODYNAMIC ANALYSES

## 9.1. Pharmacokinetic Analyses

Pharmacokinetics (PK) analyses will be based on the PKEDS. Descriptive statistics (arithmetic mean, SD, median, minimum, maximum, geometric mean, and coefficient of variation) will be presented for the concentration of *tirasemtiv* and its metabolites, and for riluzole by treatment group and study visit. The data collected from this study will be added to the population-based PK models and full covariate analyses will be performed across all studies and reported separately.

All concentrations below the lower limit of quantification will be set to zero for the purpose of calculating descriptive statistics. Additionally, a plasma concentration listing with nominal time and the actual elapsed time since the prior dose (sample time – dosing time) will be provided.

## 9.2. Pharmacokinetic/Pharmacodynamic Analyses

Pharmacodynamic (PD) analyses may be performed to explore the relationship between trough *tirasemtiv* concentration and efficacy and safety endpoints.

# 10. DATA HANDLING AND DERIVATIONS

## 10.1. Missing Data Handling for Sensitivity Analyses

### 10.1.1. Handling of Missing Primary Endpoint Data in Patients for the Primary Analysis

To minimize the potential for missing data, patients who wish to discontinue study drug will be encouraged to continue study assessments for all protocol planned visits.

## 10.2. Derivations

### 10.2.1. Time points and Duration

Duration (Days) = Stop Date – Start Date +1

Duration (Years) = (Stop Date – Start Date +1)/365.25

Time on Treatment (Days) during the Double-Blind, Placebo-Controlled Phase = Last dosing date in the Double-Blind, Placebo-Controlled Phase – first dosing date in the Double-Blind, Placebo-Controlled Phase +1.

Time since ALS symptom Onset (month) = (Date of screening – date of ALS symptom onset)/365.25*12

Time since ALS diagnosis (month) = (Date of screening – date of ALS diagnosis)/365.25*12

### 10.2.2. Baseline Measurements for Efficacy Endpoints

Baseline efficacy measurements for respiratory and skeletal muscle function endpoints are defined as the last non-missing measurements obtained prior to the first dose of *tirasemtiv*. Baselines for ALSFRS-R, the ALSAQ-5, and the Epworth Sleepiness Scale, and Caregiver Burden are defined as measurements obtained at Week -2 because they assess the current ALS disease status.

### 10.2.3. Time to Event Endpoints

The following censoring scenario will be employed: The patient will be censored at the end of the time interval of interest or at the time of early termination from the study whichever comes first. The primary analysis for time to event endpoints will utilize this censoring scenario.

### 10.2.4. Responder Endpoints

In deriving the responder endpoints, two scenarios will be considered for patients who terminate early from the study.

1. A patient will be considered as non-responder if the patient terminates early from the study and is only on the study for less than 5/6 of the time interval specified in the definition of the responder endpoint even if the patient meets the definition of the responder. E.g. if a patient is alive, free from respiratory insufficiency, and does not report decline from baseline in the percent predicted SVC while on study but is only on

study for 16 weeks, this patient is not a responder. The primary analysis for the responder endpoints will use this rule to define a responder.

2. A completer analysis for the responder endpoints will be performed as supportive analysis. Patients who are on the treatment for at least 3/4 of the time interval specified in the definition of the responder endpoint are considered as completers.

### 10.2.5. Change from the end of 24 Weeks to the end of 48 Weeks

The change from the end of 24 weeks to the end of 48 weeks is calculated and described as the measurements collected at the Week 48 visit minus the measurements collected at the Week 24 visit. Patients who terminate early from the study prior to completing the Week 24 assessment will be set to missing for this endpoint. These analyses will include patients who have measures at Week 24.

### 10.2.6. ALSFRS-R

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: speech (speech, salivation, swallowing), fine motor (handwriting, cutting food and handling utensils, dressing hygiene), gross motor (turning in bed and adjusting bedclothes, walking, climbing stairs) and respiratory (dyspnea, orthopnea, respiratory insufficiency). The total score of the ALSFRS-R cannot be calculated if answers to any of the 12 individual questions is missing.

### 10.2.7. Respiratory Function

**Percent Predicted Slow Vital Capacity (SVC)**

The best result from trials 1, 2, and 3 for slow vital capacity (SVC), measured in liters, as recorded on the eCRFs, will be used for analysis.

### 10.2.8. Skeletal Muscle Strength

**Muscle strength measurements**

The average of results from trial 1 and trial 2 for the muscle strength test using Hand Held Dynamometry (HHD) as recorded on the eCRFs, will be used for analysis.

The mega-score is calculated as the average of available percent muscle strength change from baseline of all individual muscles at any nominal time point (Shefner, 2013). Each muscle strength value is scaled as percent change from baseline by the following formula: (observed value – baseline value)/baseline value*100.

In addition, analysis restricted to muscle groups with the ability to break HHD measurements (i.e., able to overcome the resistance applied by the evaluator) will be conducted separately. Only break measurements will be used to calculate baseline and will be included in this analysis.

### 10.2.9. Quality of Life and Other Questionnaires

**ALSAQ-5**

Raw scores will be assigned to each question as follows:

0 = Never

1 = Rarely

2 = Sometimes

3 = Often

4 = Always or cannot do at all

The following domain scores are specified as below (Jenkinson, Fitzpatrick, Swash, & Levvy, 2001).

1. Physical mobility = raw score of Q1.
2. Activities of daily living/independence = raw score of Q2
3. Eating and Drinking = raw score of Q3
4. Communication = raw score of Q4
5. Emotional functioning = raw score of Q5

A total score of ALSAQ-5 is the sum of all five domain scores. The total score will be set to missing if any domain score is missing.

**Epworth Sleepiness Scale Score**

The Epworth Sleepiness Scale score is the sum of 8 item-scores. The score will be rounded up to the next whole number before calculating the sum if the score is not a whole number. If one or more item-scores are missing then the Epworth Sleepiness Scale score is invalid and will be set to missing in the analysis (Johns, 1991).

**Caregiver Burden**

The average burden score for each domain listed below will be calculated. The overall average burden score will be calculated by averaging the mean scores from each domain.

1. Time Dependence Burden
2. Developmental Burden
3. Physical Image Burden
4. Social Burden

## 10.3. Analysis Visit Windows

The analysis visit windows below assign measurements to each corresponding visit week that will be used in the analysis. Analysis visit windows are defined for measurements collected in the 48 weeks of the Double-Blind, Placebo-Controlled Phase as well as in the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase.

1. Baseline (Week -2): visit where baseline defined in Section 10.2.2 is collected.
2. Day 1: measurements collected at the randomization visit where the double-blind placebo-controlled study medication kit is dispensed.

3. 48-Week Double-Blind, Placebo-Controlled Phase:

Week 4 – Week 48: The clinical visit performed prior to the treatment discontinuation will be used as the analysis visit. Visits performed after the last dosing date will be mapped using the following analysis visit window expressed as days post-randomization.

Week 4: Day 14 – Day 42

Week 8: Day 43 – Day 70

Week 12: Day 71 – Day 98

Week 16: Day 99 – Day 126

Week 20: Day 127 – Day 154

Week 24: Day 155 – Day 196

Week 32: Day 197 – Day 252

Week 40: Day 253 – Day 308

Week 48: Day 309 – Day 350

Measurements collected at an actual nominal visit will be used when more than one measurement falls at the same visit after applying the visit window rules. If there is no actual nominal visit and two or more measurements fall in the same visit window, the measurement collected closest to the nominal visit will be used. The later measurement will be used if there are ties.

The analysis of safety assessments collected at nominal visits will use the nominal visits. No visit window will be applied except for weight. The visit windows for efficacy will be applied to weight. Unscheduled visits will not be used in analysis unless the scheduled visit is missing and the unscheduled visit falls within the scheduled visit window specified in the protocol. When an early termination visit falls on the scheduled visit, the early termination visit assessment will be performed instead of the scheduled visit. The early termination visit will be reset to its corresponding visit and used in the analysis.

4. Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase: The following visit window expressed as days post second randomization to the Double-Blind, Placebo-Controlled *Tirasemtiv* Withdrawal Phase will be applied.

Week 49: Day 7 – Day 14

Week 52: Day 21 – Day 35

# 11. COMPUTER METHODS

Statistical analyses will be performed using Version 9.4 (or newer) of SAS® on Microsoft Windows operating system or Version 9.3 on Unix system. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc. in the USA and other countries. ® indicates USA registration.

# 12. CHANGES TO ANALYSES SPECIFIED IN PROTOCOL

All changes in statistical methods that are described in the statistical analysis plan will be documented in the clinical study report.

# 13. 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 prior to the database lock and will be documented in the specification document for the statistical report of the study.

1. SAS code for the final analysis for the primary endpoint at Week 24

```
proc mixed data=work;
      class <subject> <riluzole use> <dose level> <visit> <pooled site>;
      model chg=<base> <base>*<visit> <riluzole use> <visit> <pooled site> <dose level> <dose
      level>*<visit>/solution ddfm=kr ;
      repeated <visit>/subject=<subject> type=un;
      lsmestimate <dose level>*<visit> '250vs. pbo at Week 24'
                                       [-1, 1 7] [1, 2 7],
                                       '375vs. pbo at Week 24'
                                       [-1, 1 7] [1, 3 7],
                                       '500vs. pbo at Week 24'
                                       [-1, 1 7] [1, 4 7]
                                       /e cl elsm;
      lsmestimate <dose level>*<visit> 'pbo vs. pooled tirasemtiv at Week 24'
                                       [-1, 1 7] [&c1, 2 7] [&c2, 3 7] [&c3, 4 7]
                                       /e cl elsm;
ods output LSMEstimates=lsme ;
      run;
```

This code assumes that the analysis involves four levels in treatment groups (e.g., placebo group is coded as 0 and 250, 375, and 500 mg are coded as 1, 2, and 3 respectively). Variable VISITNUM has seven categories with Day 1 coded as 1 and Week 24 coded as 7. The coefficients &c1, &c2 and &c3 for each *tirasemtiv* target dose level to be used in LSMESTIMATE statement in order to obtain the pooled *tirasemtiv* vs. placebo effect are determined by the proportion of patients evaluated in each target group among all the patients in the *tirasemtiv* group at Week 24.

Analysis at Week 48 will use a similar model where the level for VISITNUM is 10 for Week 48. Estimates from the model at Week 48 will be obtained using the following statement where the coefficients in the statement to obtained pooled *tirasemtiv* vs. placebo effects will be based on the proportion of patients in each target group among all patients in the *tirasemtiv* group at Week 48.

```
      lsmestimate <dose level>*<visit> '250vs. pbo at Week  48'
                                       [-1, 1 10] [1, 2 10],
                                       '375vs. pbo at Week  48'
                                       [-1, 1 10] [1, 3 10],
                                       '500vs. pbo at Week  48'
                                       [-1, 1 10] [1, 4 10]
                                       /e cl elsm;
      lsmestimate <dose level>*<visit> 'pbo vs. pooled tirasemtiv at Week  48'
                                       [-1, 1 10] [&c1, 2 10] [&c2, 3 10] [&c3, 4 10]
                                       /e cl elsm;
```

2. SAS code for Slope analysis

```
PROC MIXED data=work METHOD=REML;
```

```
        class <Subject> <dose level> <riluzole use> <pooled site>;
        model <chg> = <base> <dose level> <pooled site> <riluzole use> <days from base> <dose
        level>*<base> <dose level>*<days from base>/
        ddfm=kenwardroger noint;
        Random <days from base> / type=un subject=<Subject>;
        Estimate 'Slope Diff between 250mg vs. pbo' <dose level>*<days from base> -1 1
        /cl e;
        Estimate 'Slope Diff between pooled act vs. pbo' <dose level>*<days from base> -1 &c1 &c2 &c3/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. <dose level>*<riluzole use> will be included in the model for skeletal muscle function related endpoints. Coefficients to obtain the slope difference between all *tirasemtiv* target dose levels vs. placebo will be determined based on the proportion of patients in each target dose among all patients in *tirasemtiv* group and have at least one post-baseline assessment included in the model.

3. The SAS code to rank the observations by visit is as follows.

```
Proc rank data=work out=rwork nplus1;
        Var <chg>;
        Ranks r<chg>;
        By <visit>;
Run;
```

   The SAS code to rank the observations across the visits is as follows.

```
Proc rank data=work out=rwork nplus1;
        Var <chg>;
        Ranks r<chg>;
Run;
```

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

```
PROC phreg data=work;
        Class <dose level>(ref=first) <pooled site> <Riluzole use>;
        Model <response>*censor (<censor list>)=<SVC base> <dose level> <riluzole use>;
        Hazardratio <dose level>;
        Strata <pooled site>;
Run;
```

5. SAS code for the conditional logistic regression model

```
proc logistic data=work descending;
        class <dose level> <riluzole use> <pool site>/ ref=first param=glm ;
        model <resp>=<base> <dose level> <riluzole use>/link=logit ;
        strata <pool site>;
        lsmeans <dose level>/e cl ilink;
        lsmestimate mtdosen 'all tirasemtiv vs pbo' &c1 &c2 &c3 -1/e elsm cl exp;
        ods output OddsRatios=odds;
run;
```

Note this code assumes placebo is coded as 0 and 250, 375 and 500 mg are coded as 1, 2, and 3 respectively. Coefficients in the LSESTIMATE statement will be determined based on the proportion of patients in each target group among all *tirasemtiv* patients.

6. SAS code for GEE proportional odds analysis

```
proc genmod data=work;
  class <dose level> <riluzole use>;
```

```
        model <resp> = <baseline> <dose level> <riluzole use>
                                / dist=multinomial
              link=cumlogit
              aggregate=<dose level>
              type1;
  estimate 'LogOR12' dose  -1 1 / exp;
  estimate 'LogOR13' dose  -1  0  1 / exp;
  estimate 'LogOR14' dose  -1  0  0  1 / exp;
run;
```

Note this code assumes placebo is coded as 0 and 250, 375 and 500 mg are coded as 1, 2 and 3 repectively.

7. SAS code to perform WLW analysis

```
proc phreg data=work covs(aggregate);
        model <Time to onset>*<Censor>(0)=<base> trt11 - trt34
        <riluzole use> <pooled site>;
   strata <ENUM>;
   id <subjid>;
         trt11 = <trt1>*(ENum=1);
         trt12 = <trt1>*(ENum=2);
         trt13 = <trt1>*(ENum=3);
         trt14 = <trt1>*(ENum=4);

         trt21 = <trt2>*(ENum=1);
         trt22 = <trt2>*(ENum=2);
         trt23 = <trt2>*(ENum=3);
         trt24 = <trt2>*(ENum=4);

         trt31 = <trt3>*(ENum=1);
         trt32 = <trt3>*(ENum=2);
         trt33 = <trt3>*(ENum=3);
         trt34 = <trt3>*(ENum=4);

    Average 250vspbo: trt11, trt12, trt13, trt14 / e average;
    Average 375vspbo: trt21, trt22, trt23, trt24 / e average;
    Average 500vspbo: trt31, trt32, trt33, trt34 / e average;

  run;
```

TIME is time to each event from baseline. The maximum level for the composite endpoints in the exploratory analysis is 4. Enum=1 indicates first event, 2 second event, etc. All levels are populated for each subject,e.g. if a subject only experiences two events, this subject will have ENUM=3 and CENSOR=0 as well as ENUM=4 and CENSOR=0, where CENSOR=0 means censored. TRT1 is 1 when Treatment = 250 mg and 0 for all other treatment groups. Similarly TRT2=1 when treatment = 375 mg and 0 for all other treatment groups. <Time to onset> is the time from the start of the double blind, placebo-controlled dose to the time of the onset of the event.

8. 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> <week4> <week8> <week12><week 16> <week 20> <week 24> <week 32> <week 40> <week
48>;
            by <treatment>;
run;
```

Step 2: Analyze the complete data

```
PROC MIXED data=work METHOD=REML;
 class <Subject> <Treatment> <Visit> <Riluzole use> <pooled site>;
 model <chg> = <Baseline> <Baseline>*<Visit> <Treatment> <Visit>
      <Pooled Site> <Riluzole use> <Treatment>*<Visit>/
                  ddfm=kenwardroger solution covb;
 repeated <Visit> / SUBJECT=<Subject> Type=UN;
 lsmestimate <dose level>*<visit> 'pbo vs. pooled tirasemtiv at Week 24'
                              [-1, 1 7] [&c1, 2 7] [&c2, 3 7] [&c3, 4 7] /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;
```

9. The SAS code for rank-based analysis, a more rigorous randomized-based version of within visit rankings is as follows:

   Use SAS code 8 Step 1 to impute the missing data. Number of imputation is 1 and seed should be changed accordingly. Rank will be done using the complete data following the SAS code 3 to rank observations by visit.

10. The normality assumptions for the ANCOVA analysis will be assessed by residual illustration. The outpred option in the above code 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;
```

11. 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).

12. 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;
```

13. 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> <concentration> <visit>/ddfm=kenwardroger;
 Repeated <visit> /type=UN subject=<Subject>;
run;
```

14. SAS code to assess the drug-drug interaction

```
PROC MIXED data=work METHOD=REML;
 Class <Subject> <visit> <treatment>;
 Model <chg> = <treatment> <visit> <visit>*<treatment>/ddfm=kenwardroger;
 Repeated <visit> /type=UN subject=<Subject>;
 Lsmeans <visit>*<treatment>;
run;
```

15. The SAS code for Van Elteren test is as follows:

```
PROC freq data=work;
        Table <Stratification factor>*<treatment group>*<category>/cmh2 scores= MODRIDIT;
Run;
```

16. The SAS Code to evaluate the baseline characteristics factors as predictors to response using logistic regression is as follows:

```
proc logistic data=work descending;
  class <stratification factor> <treatment> <pooled site> <base factor1> <base factor 2> … <base factor k> ;
  model <response>= <treatment> <stratification factor> <pooled site> <base factor 1> <base factor 2> … <base
factor k> / selection=s;
run;
```

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# APPENDIX A. SCHEDULE OF EVENTS

| Procedure | Screening | Open-Label Phase | Double-Blind, Placebo Controlled Phase | | | | | | | | | | | Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase | | Follow-Up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Week -4 | Week -2 | Day 1 | Week 2 | Week 4 | Week 8 | Week 12 | Week 16 | Week 20 | Week 24 | Week 32 | Week 40 | Week 48 | Week 49 | Week 52 | Week 56 |
| Informed Consent | X | | | | | | | | | | | | | | | |
| Inc/Exc Criteria | X | | | | | | | | | | | | | | | |
| Demographic Data | X | | | | | | | | | | | | | | | |
| Medical History[1] | X | | | | | | | | | | | | | | | |
| Physical Examination | X | | | | | | | | | X | | | X | | | X |
| Neurological Exam | X | | | | | | | | | X | | | X | | | X |
| Ashworth Score | X | | | | X | X | X | | | X | | | X | | | X |
| Concomitant Meds | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead ECG | X | | | | | X | | | | X | | | X | | X | X |
| Vital Signs[2] | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Clinical Safety Labs[3] | X | X | | | X | X | | | | X | | | X | | X | X |
| Serum Pregnancy[4] | X | | | | | | | | | X | | | | | | |
| PK Sample | | X | X | | X | X | | X | | X | X | X | X | X | X | |
| Biomarker Sample | | | X | | | X | | X | | X | X | X | X | | | |
| ALSFRS-R | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X |
| Handgrip Strength | | X | | | X | X | X | X | X | X | X | X | X | X | X | X |
| Respiratory Assessment | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X |
| Muscle Strength | | X | | | X | X | X | X | X | X | X | X | X | X | X | X |
| ALSAQ-5 | | X | | | | | X | | | X | | | X | | | X |
| Epworth Sleepiness Scale | | X | | | | | X | | | X | | | X | | | X |
| Suicidality Assessment | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Caregiver Burden | | X | | | | | | | | X | | | X | | | X |
| Falls Assessment | | X | X | X | 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 |
| Randomization | | | X | | | | | | | | | | X | | | |
| Study Drug Dosing | | X | X | X | X | X | X | X | X | X | X | X | X | X | | |
| Riluzole Dosing[5] | | X | X | X | X | X | X | X | X | X | X | X | X | X | | |
| Phone Contact | | X[6] | | | | | | | | | | | | | | |

1 Includes smoking history
2 Weight obtained at each visit
3 TSH only at screening visit
4 Serum pregnancy test only for females of child bearing potential
5 Riluzole dosing if applicable for patients currently taking riluzole
6 Phone contact with patient after 7 days of open-label *tirasemtiv*

## APPENDIX B. TABLE OF THE REGIONAL SITE POOLING FOR FAS

# VITALITY-ALS Regional Site Pooling for FAS

| Pooled Site | Site ID | State/Province/Country | Number of Patients in the FAS |
|---|---|---|---|
| 1 | 001013 | OR | 7 |
| 1 | 001014 | CA | 6 |
| 1 | 001016 | CA | 8 |
| 1 | 001018 | CA | 3 |
| 1 | 001019 | CO | 2 |
| 1 | 001023 | WA | 5 |
| 1 | 001026 | CA | 6 |
| 1 | 001044 | CA | 4 |
| 1 | 001050 | OR | 1 |
| 1 | 001051 | AZ | 7 |
| 1 | 001054 | CA | 2 |
| | | Subtotal | 51 |
| 2 | 001003 | KS | 7 |
| 2 | 001005 | MO | 7 |
| 2 | 001007 | TX | 10 |
| 2 | 001009 | TX | 5 |
| 2 | 001033 | MO | 3 |
| 2 | 001041 | TX | 14 |
| | | Subtotal | 46 |
| 3 | 001028 | OH | 8 |
| 3 | 001030 | IA | 7 |
| 3 | 001032 | MN | 6 |
| 3 | 001036 | MI | 4 |
| 3 | 001037 | WI | 4 |
| 3 | 001042 | NE | 2 |
| 3 | 001043 | IN | 6 |
| 3 | 001046 | MI | 8 |
| 3 | 001053 | IL | 5 |
| | | Subtotal | 50 |
| 4 | 001004 | WV | 6 |
| 4 | 001006 | PA | 8 |

| Pooled Site | Site ID | State/Province/Country | Number of Patients in the FAS |
|---|---|---|---|
| 4 | 001008 | MD | 11 |
| 4 | 001015 | PA | 7 |
| 4 | 001022 | VA | 3 |
| 4 | 001035 | DC | 4 |
| 4 | 001039 | TN | 9 |
| 4 | 001048 | PA | 3 |
| | | Subtotal | 51 |
| 5 | 001010 | NC | 12 |
| 5 | 001011 | FL | 11 |
| 5 | 001012 | FL | 14 |
| 5 | 001021 | NC | 10 |
| 5 | 001024 | FL | 7 |
| 5 | 001040 | GA | 5 |
| 5 | 001047 | NC | 7 |
| | | Subtotal | 66 |
| 6 | 001001 | NY | 2 |
| 6 | 001002 | MA | 4 |
| 6 | 001017 | NY | 10 |
| 6 | 001025 | CT | 21 |
| 6 | 001027 | NY | 7 |
| 6 | 001031 | NH | 4 |
| 6 | 001045 | MA | 3 |
| | | Subtotal | 51 |
| 7 | 002061 | Quebec | 38 |
| 7 | 002063 | Quebec | 7 |
| 7 | 002066 | Quebec | 10 |
| | | Subtotal | 55 |
| 8 | 002060 | Ontario | 17 |
| 8 | 002064 | Newfoundland | 2 |
| 8 | 002065 | Alberta | 8 |
| 8 | 002067 | Nova Scotia | 1 |
| 8 | 002068 | Alberta | 11 |
| 8 | 002070 | Ontario | 7 |
| 8 | 002071 | Ontario | 7 |
| | | Subtotal | 53 |
| 9 | 031089 | NLD | 4 |

| Pooled Site | Site ID | State/Province/Country | Number of Patients in the FAS |
|---|---|---|---|
| 9 | 032010 | BEL | 11 |
| 9 | 033091 | FRA | 4 |
| 9 | 033093 | FRA | 4 |
| 9 | 033094 | FRA | 4 |
| 9 | 033095 | FRA | 1 |
| 9 | 033096 | FRA | 2 |
| 9 | 033097 | FRA | 3 |
| 9 | 044080 | GBR | 2 |
| 9 | 044081 | GBR | 2 |
| 9 | 044082 | GBR | 1 |
| 9 | 044099 | GBR | 1 |
| 9 | 049086 | DEU | 14 |
| 9 | 049087 | DEU | 4 |
| 9 | 049088 | DEU | 6 |
| 9 | 353090 | IRL | 12 |
| | | Subtotal | 75 |
| 10 | 034098 | ESP | 28 |
| 10 | 039030 | ITA | 8 |
| 10 | 039031 | ITA | 19 |
| 10 | 039032 | ITA | 5 |
| 10 | 351020 | PRT | 3 |
| | | Subtotal | 63 |
| | | Grand Total | 561 |