PROTOCOL CY 4031

A Phase 3, 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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Study Site	Multi-National

INVESTIGATOR PROTOCOL AGREEMENT PAGE

I agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by Cytokinetics.
- Not to amend the protocol without agreement, prior review, and written approval from the Institutional Review Board (IRB), Research Ethics Board (REB), or Ethics Committee (EC), except where necessary to eliminate an immediate hazard to the patients.
- That I am thoroughly familiar with the appropriate use of the investigational product(s), as described in this protocol and any other information provided by the Sponsor including, but not limited to, the following: the current Investigator's Brochure (IB) or equivalent document.
- That I am aware of, and will comply with, "Good Clinical Practices" (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the Cytokinetics investigational product(s) and of their study-related duties and functions as described in the protocol.
- That I have been informed that certain regulatory authorities require the Sponsor to obtain and supply, as necessary, details about the Investigator's ownership interest in the Sponsor or the investigational product, and more generally about his/her financial ties with the Sponsor. Cytokinetics will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

Agree to supply Cytokinetics with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children);

Agree to promptly update this information if any relevant changes occur during the course of the study and for 1 year following completion of the study; and

Agree that Cytokinetics may disclose any information it has about such ownership interests and financial ties to regulatory authorities.

Investigator Name: _____

Investigator Signature: _____ Date: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Explanation
AE	adverse event
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase (alanine transaminase)
ALSAQ-5	ALS Assessment Questionnaire Short Form
ALSFRS-R	ALS Functional Rating Scale-Revised
AM	ante meridiem (morning)
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AST	aspartate aminotransferase (aspartate transaminase)
AUC_{inf}	area under the concentration-time curve (extrapolated to infinity)
AUC	area under the plasma concentration-time curve (during dosing interval)
BID	twice a day
BiPAP	bilevel positive airway pressure
BMI	body mass index
CBC	Complete blood count (hematology clinical laboratory evaluations)
CFR	Code of Federal Regulations
CI	confidence interval
CNS	central nervous system
CPAP	continuous positive airway pressure
CPK	creatine phosphokinase
C_{max}	maximum observed plasma concentration
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	pre-dose plasma concentration
CYP	cytochrome P450
DMC	Data Monitoring Committee
DPS	diaphragm pacing system
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FAS	full analysis set
FDA	Food and Drug Administration

Abbreviation	Explanation
GCP	Good Clinical Practices
GI	gastrointestinal
GLP	Good Laboratory Practices
HEK	human embryonic kidney
hERG	human ether-à-go-go related gene
HHD	hand-held dynamometry
IB	Investigator's Brochure
IC50	half maximal inhibitory concentration
ICF	informed consent form
ICH	International Council on Harmonisation
IND	investigational new drug
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System
K_I	inactivation constant
k_{inact}	maximum rate constant for inactivation
LFT	liver function tests
LSM	least squares mean
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
MTD	maximum tolerated dose
MVIC	Maximum Voluntary Isometric Contraction
MVV	Maximum Voluntary Ventilation
m^2	meters squared
NADPH	nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
NIPPV	non-invasive positive pressure ventilation
NOAEL	no observed adverse effect level
NYHA	New York Heart Association
PD	pharmacodynamic
PI	Principal Investigator
PK	pharmacokinetic(s)
PKEDS	pharmacokinetic evaluable data set

Abbreviation	Explanation
PM	post meridiem (evening)
PPS	per protocol set
QD	once daily
REB	Research Ethics Board
SAE	serious adverse event
SAS	safety analysis set
SD	standard deviation
SE	standard error
SNIP	Sniff Nasal Inspiratory Pressure
SVC	slow vital capacity
t_{max}	time to maximum plasma concentration
TDD	total daily dose
TEAE	treatment-emergent adverse event
TnC	troponin C
TSH	thyroid stimulating hormone
UA	urinalysis
ULN	upper limit of normal
WHO	World Health Organization

1. INTRODUCTION

1.1. Background on *Tirasemtiv*

Tirasemtiv (formerly CK-2017357) is a novel small molecule activator of fast skeletal muscle troponin, intended to improve skeletal muscle function in disease states associated with muscular weakness or fatigue, including amyotrophic lateral sclerosis (ALS), without affecting the structure of muscle itself. *Tirasemtiv* selectively binds to the fast skeletal muscle troponin complex and slows the rate of calcium release from troponin C (TnC). This increases the affinity of TnC for calcium and thus sensitizes the sarcomere to calcium. *Tirasemtiv* is selective for fast skeletal muscle troponin with little effect on slow skeletal muscle troponin and no effect on cardiac muscle troponin. By sensitizing the fast skeletal muscle troponin complex to calcium, *tirasemtiv* shifts the calcium-force relationship of muscle fibers leftward, amplifying the response of muscle to submaximal nerve stimulation and thereby increasing muscle force. *Tirasemtiv* decreases muscle fatigability in several preclinical models of exercise performance, presumably by reducing the energetic requirements of calcium cycling during muscle contraction (Russell, Hartman et al. 2012). In a transgenic mouse model of ALS with functional deficits, single doses of *tirasemtiv* significantly increased submaximal isometric force, forelimb grip strength, grid hang time, and running performance on a rotating rod. Additionally, diaphragm force and tidal volume were significantly higher (Hwee, Kennedy et al. 2014). Acute and reversible clinical signs of intolerance were the dose-limiting toxicities in preclinical testing. The pharmacological profile of *tirasemtiv* is unique in that it is a direct and selective functional activator of fast skeletal muscle; as such, it could benefit patients with a wide variety of disorders characterized by muscle weakness or fatigue.

In a Phase 2b study that enrolled 711 patients with ALS (CY 4026, also known as BENEFIT-ALS), 12 weeks' treatment with *tirasemtiv* was associated with statistically significant and potentially clinically meaningful reductions in the decline in slow vital capacity (SVC) versus placebo (see Section 1.4.2). This observation is the basis for the current study, CY 4031.

1.2. Rationale for *Tirasemtiv* in the Potential Treatment of Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis, or ALS, is a disease of the nerve cells in the brain and spinal cord that control voluntary muscle movement. In ALS, progressive death of motor neurons leads to denervation of skeletal muscles. Surviving motor units attempt to compensate for dying ones by innervating more muscle fibers (a process called sprouting) but are only partially successful (Kiernan, Vucic et al. 2011). Over time, progressive denervation and its consequent skeletal muscle atrophy lead to weakness, fatigue, and eventually complete paralysis and death, primarily from respiratory complications.

No curative therapies for ALS exist. Rilutek® (riluzole, Sanofi-Aventis U.S. LLC) is one of two medications approved for the treatment of ALS, and has a modest benefit on survival (Lacomblez, Bensimon et al. 1996). The other approved medication, Nuedexta®, has a specific effect on emotional lability, a symptom experienced by a minority of ALS patients. Two interventions that contribute greatly to the overall welfare and survival of ALS patients are the use of enteral feeding and ventilatory support.

To date, there are no available treatments that can improve skeletal muscle function, and in particular respiratory function. Because *tirasemtiv* has been demonstrated both to amplify skeletal muscle force production in response to diminished neuronal input and to delay the onset and reduce the magnitude of skeletal muscle fatigue during repeated or sustained efforts, it may be useful in the treatment of patients with ALS.

1.3. Overview of *Tirasemtiv* Nonclinical Studies

A brief summary of the nonclinical safety and pharmacokinetics (PK) of *tirasemtiv* is provided in this section. Additional information concerning nonclinical assessments of the pharmacology, PK, and safety of *tirasemtiv* is available in the Investigator's Brochure (IB).

Tirasemtiv was evaluated in a series of Good Laboratory Practices (GLP) preclinical safety pharmacology and toxicology studies, including single- and repeat-dose toxicity studies performed in Sprague-Dawley rats up to 26 weeks and beagle dogs up to 39 weeks, teratology studies in rats and rabbits, single-dose phototoxicity studies in rats, and a core battery of genotoxicity tests.

In rats, following administration of *tirasemtiv* for 26 weeks at dose levels of 0, 5, 15 and 30 mg/kg/day, target organ effects were observed at levels of 15 and 30 mg/kg/day and consisted of fibrosis and/or atrophy of the mandibular salivary glands and hepatocellular hypertrophy of the liver. The finding in the liver was fully recovered and the findings in the salivary glands were partially recovered at 30 mg/kg/day following the recovery period. The no observed adverse effect level (NOAEL) in rats was considered to be 15 mg/kg/day in this study. The exposures at the end of the study attained at the NOAEL are shown in Table 1 below.

In dogs, administration of *tirasemtiv* for 39 weeks at dose levels of 0, 6, 20 and 50 mg/kg/day resulted in adverse but reversible clinical signs at 50 mg/kg/day, non-adverse effects on body weight at all dose levels, and a decrease in food consumption at the high dose. *Tirasemtiv* caused partially reversible increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at 50 mg/kg/day, with a reversible increased total bilirubin in females at 50 mg/kg/day and a reversible increase in creatine kinase and aldolase activities in individual males and females at 50 mg/kg/day. The NOAEL in dogs was considered to be 20 mg/kg/day in this study. The exposures at the end of the study attained at the NOAEL are shown in Table 1 below.

Table 1: Exposure to *Tirasemtiv* at the NOAEL in Rat and Dog

	26-Week Rat Toxicity Studies (NOAEL)		**39-Week Dog Toxicity Studies (NOAEL)**	
	M	**F**	**M**	**F**
Dose (mg/kg/day)	15	15	20	20
C_{max} (µg/mL)	25.1	46.5	27.0	20.9
AUC (µg•h/mL)	228.5	589.0	128.8	80.6

There was no evidence of genotoxicity in bacterial reverse mutation, human lymphocyte chromosomal aberration, or rat micronucleus assays. There was no evidence of phototoxicity in single-dose studies in rat or of embryolethality, fetotoxicity, or teratogenicity in rats and rabbits. *Tirasemtiv* was observed to cause a reversible blockade of the hERG channel in human

embryonic kidney (HEK) cells *in vitro* at 250 µM, but was less than 50% inhibitory. Findings in cardiovascular, central nervous system (CNS), and respiratory safety pharmacology studies of *tirasemtiv* were not considered adverse because of their mild severity and/or limited duration.

The potential of *tirasemtiv* to inhibit cytochrome P450 (CYP) enzymes (CYP1A2, 2A6, 3A4, 2B6, 2C8, 2C9, 2C19, 2D6, and 2E1) was studied *in vitro* in human liver microsomes at concentrations up to 100 - 200 µM. Both direct and time-dependent inhibitions were assessed. *Tirasemtiv* did not inhibit CYP3A4, 2D6, 2A6, or 2E1 with half maximal inhibitory concentrations (IC_{50}s) exceeding the highest concentrations tested. *Tirasemtiv* was found to be a direct and time-dependent inhibitor of multiple CYP450 isoforms (CYP1A2, 2B6, 2C8, 2C9, and 2C19). Kinetic parameters for the time-dependent inhibition, including the time-dependent IC_{50} (inhibitory constant following 20 or 30 minute pre-incubation with microsomes + NADPH), maximum rate constant for inactivation (k_{inact}) and inactivation constant (K_I), are shown in Table 2.

Table 2: Estimated IC_{50}, k_{inact}, and K_I Values for *Tirasemtiv* Time-Dependent Inhibition

CYP Isoform	Time-Dependent IC_{50} (µM)	k_{inact} (min^{-1})	K_I (µM)
CYP1A2	5.5	0.031	1.9
CYP2B6	21	0.035	26
CYP2C8	80	0.057	46
CYP2C9	69	0.021	34
CYP2C19	4	0.051	11

1.4. Overview of *Tirasemtiv* Clinical Studies

1.4.1. Phase 1 and Phase 2a Studies

Tirasemtiv has been evaluated in three completed Phase 1 clinical trials in healthy volunteers (CY 4011, CY 4012 and CY 4013). Three Phase 2a clinical trials (CY 4021, CY 4024, and CY 4025) have been conducted in patients with ALS. A Phase 2a clinical trial in patients with claudication secondary to peripheral artery disease (CY 4022) and a Phase 2a clinical trial in patients with myasthenia gravis (CY 4023) have also been completed. The Phase 1 and Phase 2a clinical studies are summarized in Table 4. Additional details are available in the IB.

1.4.2. Phase 2b Clinical Trial in Patients with ALS (CY 4026; BENEFIT-ALS)

CY 4026, also known as BENEFIT-ALS (**B**linded **E**valuation of **N**euromuscular **E**ffects and **F**unctional **I**mprovement with ***T**irasemtiv* in **ALS**), was a Phase 2b clinical trial in which 711 patients with ALS were enrolled from 73 centers in North America and Western Europe into open-label treatment with *tirasemtiv* 125 mg twice a day. Subsequently, patients who completed one week of open-label treatment were randomized 1:1 to double-blind treatment with either *tirasemtiv* (titrated at weekly intervals to each patient's maximum tolerated dose ≤ 250 mg twice daily) or placebo for 12 weeks. Clinical assessments were made at baseline (i.e., prior to treatment with open-label *tirasemtiv*), after 4, 8, and 12 weeks of double-blind treatment, and one

and four weeks after the last dose of double-blind study drug. Safety and efficacy were assessed among the 295 patients randomized to placebo who received at least one dose of double-blind study drug and the 301 patients randomized to *tirasemtiv* who received at least one dose of double-blind study drug.

The primary endpoint in BENEFIT-ALS, the mean change from baseline in the ALS Functional Rating Scale in its revised form (ALSFRS-R), was not statistically different between treatment groups. The least squares mean (LSM) changes from baseline were −2.40 in the placebo group and −2.98 in the *tirasemtiv* group. The LSM ± standard error (SE) difference between treatment groups (i.e., *tirasemtiv* response minus placebo response) was −0.58 ± 0.366 (95% confidence interval (CI): −1.30, 0.14; p = 0.114). Although BENEFIT-ALS did not achieve its primary efficacy endpoint, two pre-specified secondary endpoints, both reflective of skeletal muscle function, were positively impacted.

Treatment with *tirasemtiv* resulted in a statistically significant and potentially clinically meaningful reduction in the decline of vital capacity (measured as SVC) which assesses strength of the skeletal muscles responsible for breathing. The percent predicted SVC declined -3.12 percentage points (±0.90) from the baseline value over 12 weeks on *tirasemtiv* compared to a decline of -8.66 percentage points (±0.80) in the placebo group (p= <0.0001). This pre-specified secondary efficacy endpoint declined less on *tirasemtiv* than on placebo at each assessment time point and this difference persisted throughout 28 days after discontinuation of double-blind treatment (Figure 1, Table 3). Vital capacity has been shown to be an important predictor of disease progression and survival in prior clinical trials of patients with ALS.

Figure 1: Changes from Baseline in Percent Predicted Slow Vital Capacity in BENEFIT-ALS

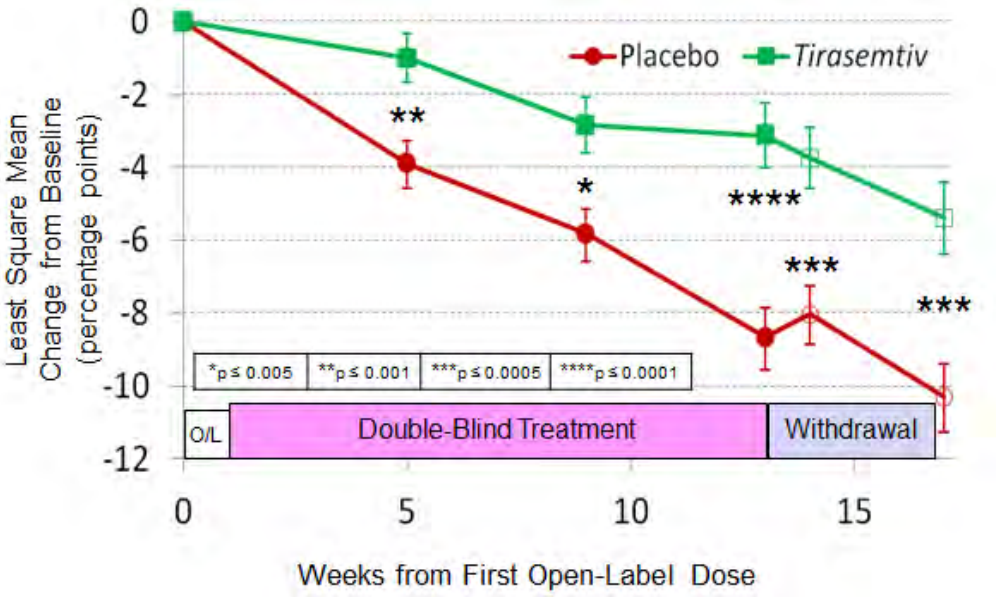
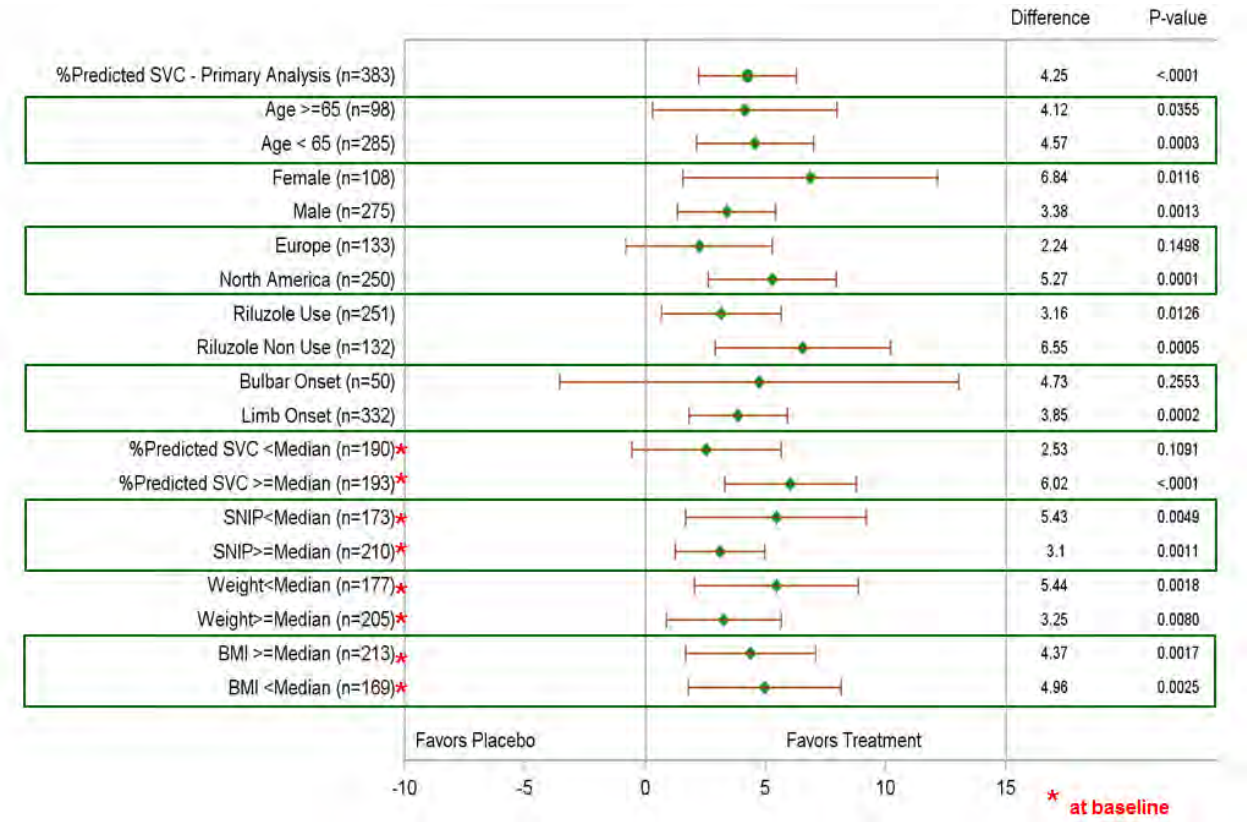


Table 3: Percent Predicted Slow Vital Capacity in BENEFIT-ALS

Slow Vital Capacity	**Placebo (n = 210)**	***Tirasemtiv* (n = 178)**	**All (N = 388)**
Baseline (prior to the one week Open-Label phase) (% Predicted, mean ± Standard Deviation [SD])	89.7 (17.2)	85.7 (19.3)	87.8 (18.3)
Time Point during the Double-Blind Treatment	**Changes from Baseline (LSM ± SE)**		**p-value**
Week 4	-3.89 (0.62)	-0.99 (0.68)	0.001
Week 8	-5.81 (0.68)	-2.85 (0.77)	0.004
Week 12	-8.66 (0.80)	-3.12 (0.90)	<0.0001
	Slope of decline (Percentage Points per day)		
Baseline to Week 12	-0.0905	-0.0394	0.0006
	Changes from Baseline (LSM ± SE)		
Week 13 (1 week after last DB dose)	-8.03 (0.77)	-3.75 (0.84)	0.0002
Week 16 (4 weeks after last DB dose)	-10.30 (0.90)	-5.39 (0.98)	0.0002

As shown in Figure 2, *tirasemtiv* reduced the decline in SVC compared to placebo regardless of age, gender, riluzole use, or body mass index (BMI). Subgroups with the largest and most significant differences in SVC on *tirasemtiv* versus placebo (change from baseline to mean SVC after eight and 12 weeks of double-blind treatment) were: females (6.84%, $p = 0.012$); non-riluzole users (6.55%, $p = 0.0005$); and patients with baseline SVC ≥ median at baseline (6.02%, $p < 0.0001$).

Figure 2: Change from Baseline to Average after 8 and 12 Weeks in Percent Predicted Slow Vital Capacity



The results of other secondary endpoints assessed in this trial were mixed. The Muscle Strength Mega-Score, a measure of strength based on the percent change from baseline from several muscle groups in each patient, declined more slowly on *tirasemtiv* versus placebo ($p = 0.016$ for the difference in slope of decline); however, there were no differences at any time point that reached statistical significance. The rate of decline for Sniff Nasal Inspiratory Pressure (SNIP) was not statistically significantly different between *tirasemtiv* and placebo ($p = 0. 21$); however, SNIP decreased statistically significantly more on *tirasemtiv* compared with placebo at four and 12 weeks (p values at 4, 8, and 12 weeks were 0.012, 0.066, 0.050, respectively). No differences in Maximum Voluntary Ventilation (MVV) and Hand Grip Fatigue were observed on *tirasemtiv* versus placebo.

Serious adverse events (SAEs) during double-blind treatment were more frequent on *tirasemtiv* than on placebo (9.0% vs. 5.4%). The most common SAE was respiratory failure, which occurred in one patient on *tirasemtiv* and three patients on placebo, while confusional state and delirium occurred in two patients on *tirasemtiv* and no patients on placebo. Of patients receiving at least one dose of double-blind study drug, more patients on *tirasemtiv* withdrew from the trial following randomization than on placebo (97 vs. 26 patients, respectively). Adverse events (AEs) more common on *tirasemtiv* than on placebo (> 10% difference) were dizziness (50.8% vs. 19.7%), fatigue (33.2% vs. 14.2%), and nausea (21.9% vs. 7.8%).

Patients on *tirasemtiv* lost more weight than patients on placebo (change from baseline to Week 12: -1.70 kg vs. -0.79 kg; $p = 0.006$). Weight loss was significantly greater in patients

with gastrointestinal (GI) AEs (e.g., nausea and decreased appetite) on either treatment. However, such AEs occurred more frequently on *tirasemtiv* than on placebo (43.5% vs. 25.8%).

The statistically significant effect to reduce the decline in vital capacity observed in BENEFIT-ALS is a unique finding that has not been observed in any prior, sizable clinical trial in ALS patients. Vital capacity is a quantitative measure of respiratory muscle function in ALS and has been shown to predict disease progression in prior clinical trials. The significant results on SVC demonstrated in BENEFIT-ALS show that *tirasemtiv* has a biological effect. The cardinal symptom of ALS that directly affects survival is weakness of the skeletal muscles necessary for respiration. Therefore, the significant reduction in the decline in vital capacity seen in this trial over 12 weeks is clinically relevant to patients with ALS. Furthermore, the difference in vital capacity (measured by SVC) between *tirasemtiv* and placebo was maintained four weeks after discontinuation of double-blind treatment. This suggests that *tirasemtiv* may have a durable impact on this measure of respiratory function in patients with ALS. These effects, if persistent over longer periods of time, suggest that *tirasemtiv* may reduce progressive respiratory decline in patients with ALS. Consequently, the current Phase 3 protocol, CY 4031, has been developed with the objective to confirm and extend the potentially beneficial effects of *tirasemtiv* on SVC and muscle strength observed in BENEFIT-ALS.

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Healthy Male Volunteers Phase 1 (CY 4011A)	57	• Double-blind, placebo-controlled; parallel dosing; *tirasemtiv* vs. placebo • Determine safety, tolerability and PK of increasing single doses • Determine maximum tolerated dose (MTD) and plasma concentration	• Single doses up to 2500 mg administered – Solid active pharmaceutical ingredient in capsule: 20 mg to 1250 mg – Liquid suspension: 640 mg to 2500 mg • MTD determined to be 2000 mg; mean C_{max} = 29.24 μg/mL • Median T_{max} 5.0 hr for both formulations • Terminal $t_{1/2}$ averaged 12.2 and 11.5 hours for the solid and liquid formulation, respectively • Dose proportional increase in AUC_{inf} over the dose range of 20 to 2500 mg • *tirasemtiv* was generally well tolerated; no SAEs	07 May 2009 to 17 Feb 2010
Healthy Male Volunteers Phase 1 (CY 4011B)	12	• Double-blind, randomized, placebo-controlled • 4-period, cross-over; single doses of 250, 500, 1000 mg and placebo in random order • Assess pharmacodynamic (PD) effects • Relate any effects observed to associated plasma concentrations	• Statistically significant increases versus placebo in peak force generated by the tibialis anterior muscle during transcutaneous stimulation of the common peroneal nerve that were related to nerve stimulation frequency and to *tirasemtiv* dose and plasma concentration • *Tirasemtiv* was generally well tolerated; no SAEs	07 Oct 2009 to 21 Dec 2009

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv (Continued)*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Healthy Male Volunteers Phase 1 (CY 4012)	24	• Double-blind, randomized, placebo-controlled • Once daily dosing for 7 days • Two cohorts, parallel dosing: – *tirasemtiv* 250 mg vs. placebo – *tirasemtiv* 375 mg vs. placebo • After multiple doses to steady-state: – Assess safety and tolerability – Evaluate the PK profile	• Dose proportional C_{max} and AUC_{24hr} • Modest (~70%) accumulation from single-dose to steady-state – Mean C_{max} of 6.34 and 8.22 µg/mL after single doses of 250 mg and 375 mg, respectively – Mean C_{max} of 9.13 and 13.39 µ/mL after dosing to steady-state with 250 mg QD and 375 mg QD, respectively • Mean $t_{1/2}$ of 8.99 hr and 12.28 hr after dosing to steady-state with 250 mg QD and 375 mg QD, respectively • Systemic exposures were high; inter-subject variability was low • *Tirasemtiv* was generally well tolerated; no SAEs	28 Oct 2009 to 17 Dec 2009

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv (Continued)*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Healthy Male or Female Volunteers Phase 1 (CY 4013)	39	• Open-label, drug-drug interaction (DDI) and food effect study (2 part study) Part A • steady-state administration of *tirasemtiv* on the PK of single doses of riluzole Part B • steady-state administration of *tirasemtiv* on the PK of single doses of a warfarin + rosiglitazone cocktail • effect of food on a single dose of *tirasemtiv* • safety and tolerability of multiple doses of *tirasemtiv*	Part A • Steady-state *tirasemtiv* (250 mg) raised the mean C_{max} of riluzole approximately 2.04-fold, mean AUC_{inf} approximately 3.77-fold compared with riluzole alone • Mean $t_{1/2}$ of riluzole increased from 7.52 hours to 14.72 hours in the presence of steady-state *tirasemtiv* • Co-administration of *tirasemtiv* reduced the inter-subject variability of riluzole C_{max} and clearance Part B • Analysis of PK data from Part B of the study indicated that the AUC_{inf} for rosiglitazone and both S-warfarin and R-warfarin increased approximately 2-fold in the presence of steady-state plasma *tirasemtiv* achieved by daily dosing at a dosage of 250 mg once daily • Food had no effect on $t_{max,}$ or AUC • Administration of a high-fat meal increased the C_{max} of *tirasemtiv* by approximately 55%	15 Mar 2011 to 27 Jun 2011

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv (Continued)*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Patients with ALS Phase 2a (CY 4021)	67	• Double-blind, randomized, placebo-controlled • 3-period, crossover study • Single doses of *tirasemtiv* (250 mg, 500 mg) and placebo	• Single doses of *tirasemtiv* were safe and generally well tolerated • Dizziness, which was generally mild, was the most frequently reported and most clearly dose-related adverse event • Both patients and investigators perceived a dose- and concentration-dependent improvement in the patients' overall status at 6 hours after dosing • Statistically significant improvement in Maximum Voluntary Ventilation (MVV) at 6 and 24 hours after a single 500 mg dose; Sniff Nasal Inspiratory Pressure (SNIP) also trended to increase • Trend to increase in sub-maximum grip strength endurance • Small but statistically significant increases in Maximum Voluntary Isometric Contraction (MVIC) strength of some but not all muscles studied	29 Mar 2010 to 2 Nov 2010

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv (Continued)*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Patients with Claudication Phase 2a (CY 4022)	61	• Double-blind, randomized, placebo-controlled • 3-period cross-over study • Single doses of *tirasemtiv* (375 mg, 750 mg) and placebo; the protocol was amended to reduce the 750 mg dose to 500 mg	• Single doses of 375mg and 500 mg were safe and generally well tolerated • Two patients experienced SAEs including severe dizziness, prompting a protocol amendment to reduce the 750 mg dose to 500 mg • Statistically significant and dose-related increases in total calf muscle work assessed by heel raise testing • Statistically significant and dose-related decreases in 6 minute walk distance, possibly due to AEs such as dizziness that interfered with performance of the test	28 May 2010 to 31 Mar 2011

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv (Continued)*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Patients with ALS Phase 2a (CY 4024)	49	• Double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of 14 days dosing of *tirasemtiv* without and with the concomitant administration of riluzole • 14 days of once daily oral dosing with *tirasemtiv* or placebo • Four parallel treatment groups – Placebo – *tirasemtiv* 125 mg – *tirasemtiv* 250 mg – *tirasemtiv* 375 mg • Part A (no riluzole): 7-day washout of riluzole followed by 14 days of *tirasemtiv* or placebo • Part B: All patients took riluzole 50 mg once daily for 7 days before and during the 14 days of double-blind, once daily *tirasemtiv* or placebo	• 49 patients treated – Placebo: 6 Part A, 7 Part B – *tirasemtiv* 125 mg: 6 Part A, 6 Part B – *tirasemtiv* 250 mg: 6 Part A, 6 Part B – *tirasemtiv* 375 mg: 6 Part A, 6 Part B • *Tirasemtiv* appeared to be safe and well tolerated at once daily doses of 125 mg, 250 mg, and 375 mg daily for 14 days • Plasma concentrations of *tirasemtiv* were unaffected by co-administration with riluzole, while plasma concentrations of riluzole approximately doubled during co-administration with *tirasemtiv* • AEs and clinical outcome measures during treatment with *tirasemtiv* appeared similar with or without co-administration of riluzole 50 mg daily • The most frequently reported adverse event was dizziness. Most episodes were mild in intensity and began and resolved early after initiation of treatment and during continued dosing • Encouraging trends to increase the ALS Functional Rating Scale-Revised score and MVV were observed in patients treated with *tirasemtiv*	Part A: 20 Jun 2011 to 10 Nov 2011 Part B: 05 Oct 2011 to 02 Mar 2012

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv (Continued)*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Patients with ALS Phase 2a (CY 4025)	27	• Double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of 21 days dosing of *tirasemtiv* administered according to a twice-daily, dose titration regimen with the concomitant administration of riluzole • 21 days of twice daily oral dosing with *tirasemtiv* or placebo • Two parallel treatment groups – Placebo dose titration – Week 1: 1 tablet twice daily – Week 2: 1 tablet in the morning and 2 tablets in the evening – Week 3: 2 tablets twice daily – *Tirasemtiv* dose titration – Week 1: 125 mg twice daily – Week 2: 125 mg in the morning and 250 mg in the evening – Week 3: 250 mg twice daily • All patients took riluzole 50 mg once daily for 7 days before and during the 21 days of double-blind, twice-daily dose titration with *tirasemtiv* or placebo	• 27 patients treated – Placebo titration: 6 – *tirasemtiv* titration: 21 • *Tirasemtiv* administered in the twice-daily, dose titration regimen studied appeared to be safe and well tolerated • 14 of 21 patients randomized to *tirasemtiv* were titrated to *tirasemtiv* 250 mg twice daily and completed the study at that dose level • Dizziness was the most frequent adverse event, reported by 12 of 21 patients on *tirasemtiv* – Mild in 10 patients, moderate in 2 patients – Resolved during continued dosing in 6 patients • Encouraging trends to increase the ALS Functional Rating Scale-Revised score and MVV were observed in patients treated with *tirasemtiv*	18 Nov 2011 to 16 Mar 2012

Table 4: Completed Phase 1 and Phase 2a Clinical Studies with *Tirasemtiv (Continued)*

Population Phase (Study #)	N	Trial Design	Results	Start/End Start = First screened End = Last visit
Patients with Myasthenia Gravis Phase 2a (CY 4023)	32	• Double-blind, randomized, placebo-controlled • 3-period cross-over study • Single doses of *tirasemtiv* (250 mg, 500 mg) and placebo	• Single doses of *tirasemtiv* were safe and generally well tolerated • Dizziness, which was generally mild, was the most commonly reported adverse event • There were no premature terminations and no SAEs reported • Quantitative Myasthenia Gravis (QMG) score decreased in a dose-related fashion • Forced Vital Capacity (FVC) increased versus placebo at 6 hours after dosing	29 Dec 2010 to 10 Oct 2012

2. STUDY OBJECTIVES

2.1. Primary Objective

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

2.1.1. Primary Endpoint

The primary endpoint is the change from baseline to Week 24 of the double-blind, placebo-controlled phase in percent predicted SVC.

2.2. Secondary Objectives

Secondary objectives include:

- Evaluation of alternative methods to assess the effect of *tirasemtiv* versus placebo on percent predicted 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 measures of skeletal muscle function in patients with ALS

2.2.1. Secondary Endpoints

The following secondary endpoints will be analyzed in a closed testing procedure if the primary efficacy analysis is met as defined in Section 8.4.3.

- Change from baseline in the ALSFRS-R score of the three respiratory items of the ALSFRS-R (i.e., the sum of items 10, 11 and 12) at the end of 48 weeks of double-blind, placebo-controlled treatment
- Slope of mega-score of muscle strength during the 48 weeks of double-blind, placebo-controlled treatment
- 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 at the end of the 48 weeks of double-blind, placebo-controlled treatment
- 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
- Change from baseline in the ALSFRS-R total score to the end of 48 weeks of the double-blind, placebo-controlled treatment
- Time to the first use of mechanical ventilatory assistance or death during all 48 weeks of double-blind, placebo-controlled treatment

2.2.2. 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 the Statistical Analysis Plan. Additional tertiary endpoints of interest may be added to the Statistical Analysis Plan.

1. "Time to Event" analyses including:
    a. Time to the first occurrence of a decline in SVC to ≤ 50% predicted 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 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 either of 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 first three composite endpoints listed above will be analyzed with "first use of mechanical ventilatory assistance" in place of "respiratory insufficiency".

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

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. 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 mega-score of muscle strength)
    c. From the end of 24 weeks of the randomized, double-blind, placebo-controlled phase to the end of the double-blind, randomized, 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. Change in percent predicted SVC from baseline to the end of Week 12 of the double-blind, placebo-controlled phase.

3. STUDY OVERVIEW

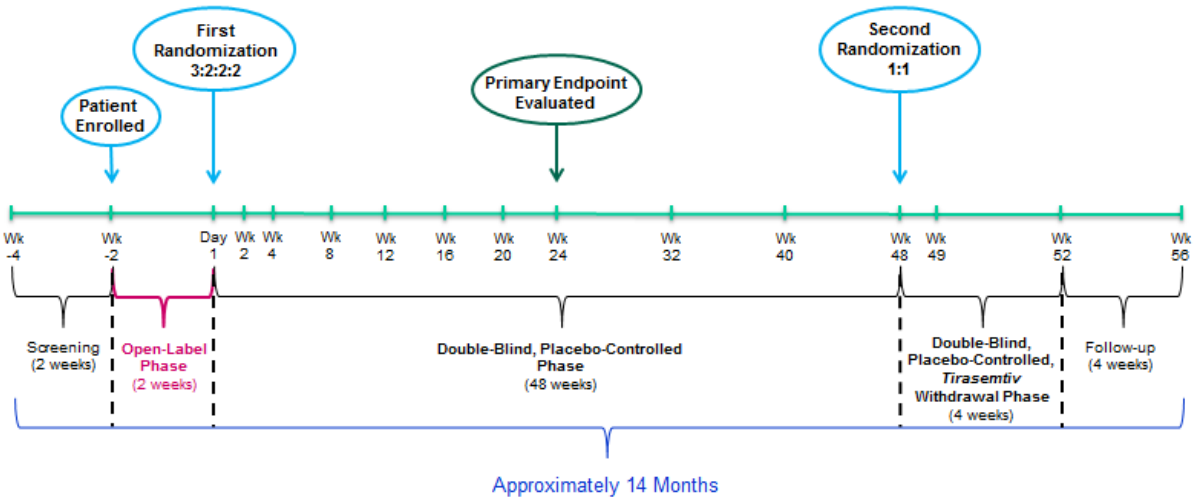
CY 4031 is a multi-national, double-blind, randomized, placebo-controlled, stratified, parallel group study with the selective fast skeletal muscle troponin activator, *tirasemtiv*, in patients with ALS who can complete two weeks of treatment with open-label *tirasemtiv* (125 mg twice daily). Both patients 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 (Figure 3); 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 dose levels of *tirasemtiv*.

Approximately 600 patients are expected to be enrolled in the open-label phase. Approximately 477 of the patients enrolled onto open-label treatment are expected to be randomized in the double-blind, placebo-controlled phase.

Figure 3: Study Design



Screening

The screening and qualification period for the study will be no more than 14 days in duration. Once patients have completed screening and are considered eligible, they will be enrolled to receive open-label *tirasemtiv* for two weeks.

Open-Label Phase

Week -2 is the first study visit after screening. At Week -2, patients will report to the study site to begin the open-label phase, which will last 14 days. During the open-label phase, patients will take 1 tablet (125 mg) of *tirasemtiv* twice daily. Patients will be contacted by phone after seven days and return to the study site after 14 days of open-label treatment.

Double-Blind, Placebo-Controlled Phase

Day 1 is the beginning of the double-blind, placebo-controlled phase, which will last approximately 48 weeks. Patients who have demonstrated adequate tolerance of *tirasemtiv* during the open-label phase will be randomized to one of four study groups (Figure 4) prior to the Day 1 procedures. Patients in Groups 2, 3, and 4 will receive double-blind *tirasemtiv* for the next 48 weeks and patients in Group 1 will receive matching placebo tablets in lieu of *tirasemtiv* (Table 5).

During the double-blind, placebo-controlled phase, additional study visits will take place at the end of Week 2, Week 4, Week 8, Week 12, Week 16, Week 20, Week 24, Week 32, Week 40, and Week 48.

Patients will not escalate their dose if signs of intolerability are present. Patients who do not tolerate a dose escalation due to symptoms believed to be due to treatment with study drug will be returned to a previously tolerated dose level. Decisions regarding dose escalation and de-escalation will be made and executed in a manner that does not compromise blinding either to treatment or to dose level.

Figure 4: First and Second Randomization

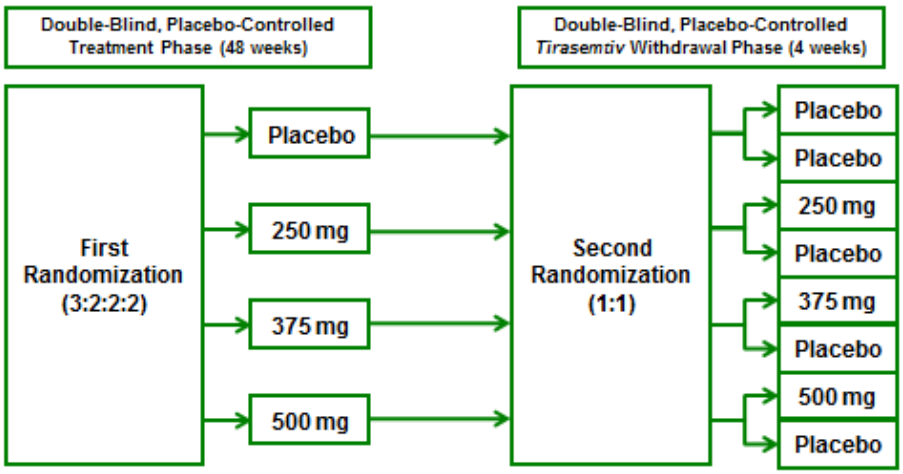


Table 5: Randomized Double-Blind Treatment Groups

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 2 placebo tablets twice daily to end of Week 48	Weeks 1 through 48 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 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 3 through 48 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 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 3 and 4 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in AM and 2 tablets of *tirasemtiv* (250 mg) in PM Weeks 5 through 48 2 tablets (250 mg) of *tirasemtiv* in AM and 2 tablets of *tirasemtiv* (250 mg) in PM
Riluzole Users Only			
Group 1 (Placebo)	**Groups 2-4 (Active Treatment Groups)**		
Riluzole tablets 50 mg riluzole (personal supply) in the morning plus blinded riluzole 50 mg in the evening	Riluzole tablets 50 mg riluzole (personal supply) in the morning plus blinded riluzole placebo in the evening		

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

Upon completion of the double-blind, placebo-controlled phase, patients will be randomized a second time (Figure 4) to placebo or to continue their current active dose level of treatment in an allocation ratio of 1:1 for the next four weeks as part of the double-blind, placebo-controlled *tirasemtiv* withdrawal phase (Table 6). Both the first and second randomizations will be stratified by riluzole use/non-use.

Table 6: Randomized Double-Blind, *Tirasemtiv* Withdrawal Treatment

Group 1 (maintained on *tirasemtiv*)	Group 2 (withdrawn to placebo)
Weeks 49 through 52 Maintained on the *tirasemtiv* dose taken during the double-blind, placebo-controlled phase	Weeks 49 through 52 Withdrawn to or maintain placebo dose in AM and PM

During the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase, additional study visits will take place at the end of Week 49 and Week 52.

A final follow-up visit will occur four weeks after completion of the double-blind, placebo-controlled *tirasemtiv* withdrawal phase at Week 56.

Riluzole

Patients who were not taking riluzole prior to study entry will be encouraged not to take riluzole until they complete study drug dosing. Patients who were not taking riluzole at study entry but who later decide to start riluzole during the dosing phase of the study will not be allowed to continue taking study drug. When patients are taking *tirasemtiv* during the study, their dose of riluzole will be reduced to 50 mg once daily in the morning as described in Section 5.8.

3.1. Estimated Study Duration

Individual patient participation will take place as follows:

Screening	2 Weeks
Open-Label Phase	2 Weeks
Double-Blind, Placebo-Controlled	48 Weeks
Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal	4 Weeks
Follow Up	4 Weeks
Total	60 Weeks

3.2. *Tirasemtiv* Dose Rationale

In the Phase 2b study of *tirasemtiv* in patients with ALS, BENEFIT-ALS, following a one week open-label run-in period at 250 mg/day, patients were titrated using weekly dose increases to their maximum tolerated dose (MTD), up to 500 mg/day. Flexible dosing was allowed to maximize tolerability. Patients tended to drop out after titration from 250 mg/day to 375 mg/day and from 375 mg/day to 500 mg/day, suggesting that a longer titration schedule may improve tolerability. Among patients on *tirasemtiv* who completed the study, approximately half were on 500 mg/day, and the remainder was divided generally equally between 375 mg/day and 250 mg/day. Analyses of the change from baseline in percent predicted SVC on *tirasemtiv* versus placebo by MTD and *tirasemtiv* exposure suggested that doses lower than 500 mg/day may still meaningfully reduce the decline from baseline in SVC in patients with ALS.

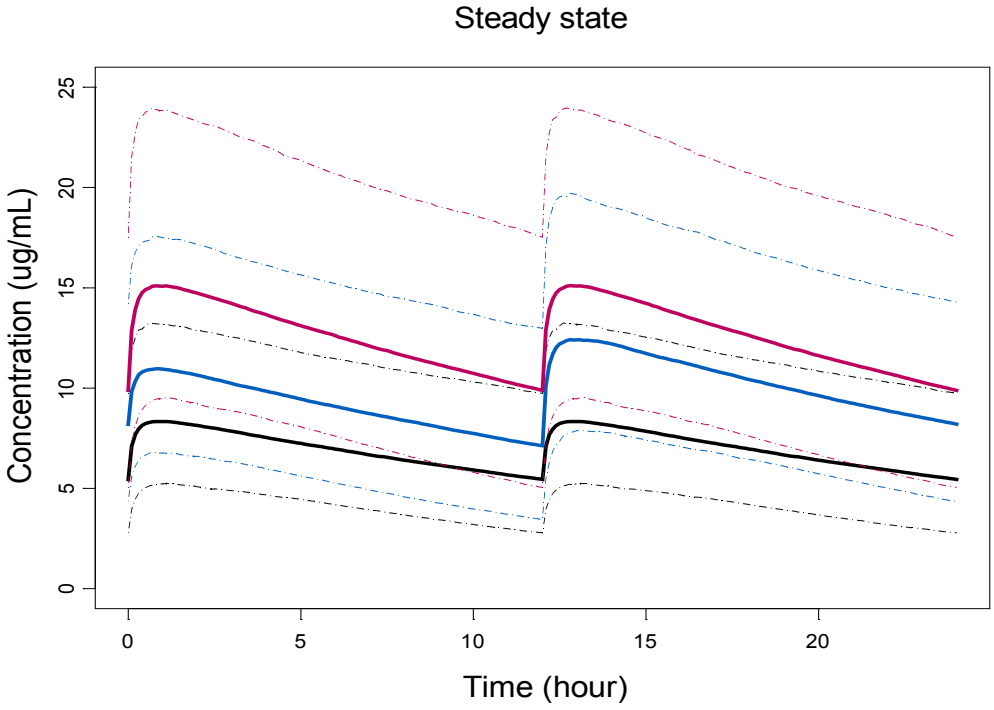
Given the data from BENEFIT-ALS, we propose to evaluate further the relationship of specific *tirasemtiv* doses to the intended effect to reduce the decline in respiratory function as measured by SVC while also attempting to improve the tolerability of *tirasemtiv* with a more gradual dose titration schedule. Thus, this study has been designed to test three separate target *tirasemtiv* doses (250 mg/day, 375 mg/day, and 500 mg/day), using a more gradual dose titration schedule than was employed in BENEFIT-ALS. Patients who discontinue drug treatment will be expected to stay in the trial and complete all scheduled assessments.

Figure 5 shows the range of *tirasemtiv* concentrations associated with the steady-state PK profiles predicted to occur in CY 4031 based on data from BENEFIT-ALS and the *tirasemtiv* population PK model. The solid lines represent the predicted average concentration-versus-time

profile and the upper and lower dashed lines represent the concentration-versus-time profiles at the 95th and 5th percentiles.

Table 7 provides the distribution of AM and PM trough *tirasemtiv* concentrations by dose level at steady-state.

Figure 5: Predicted Steady-State Pharmacokinetic Profiles for *Tirasemtiv* Target Dose Levels in CY 4031



The black lines are for 125 mg BID, the blue lines are for 125 mg AM/ 250 mg PM, and the red lines are for 250 mg BID, respectively.

Table 7: Distribution of Trough *Tirasemtiv* Concentration at Steady-State by Dose

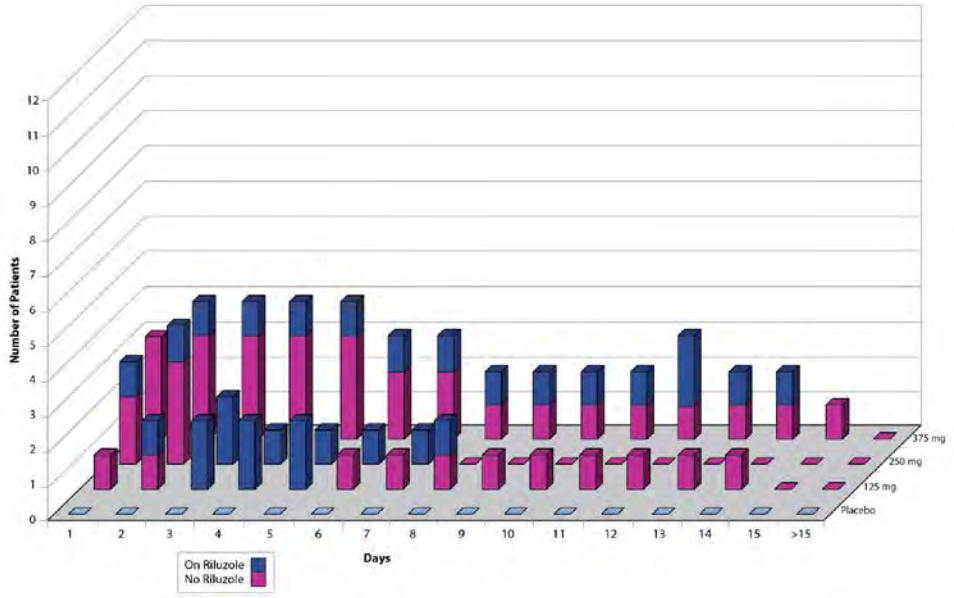
Parameters	125 mg BID	125 mg AM and 250 mg PM	250 mg BID
Trough prior to evening dose (µg/mL, median [5-95 percentile])	5.4 [2.8 - 9.7]	7.1 [3.4 - 13]	9.9 [5 - 17.5]
Trough prior to morning dose (µg/mL, median [5-95 percentile])	5.4 [2.8 - 9.7]	8.2 [4.3 - 14.3]	9.9 [5 - 17.6]
Trough prior to evening dose % of patients below 5/6/7/8 µg /ml	39.8/60.3/75.9/86	18/32.4/47.6/61.7	4.9/9.9/18/27.8
Trough prior to evening dose % of patients above 13/14/15/16 µg /ml	0.4/0.3/0.2/0.2	5/2.8/1.8/1.2	22.2/16.4/12.1/9.2

3.3. Rationale for the Open-Label Lead-In Phase Prior to Randomization

The open-label treatment phase for all patients prior to randomization to double-blind active *tirasemtiv* versus placebo has been incorporated into the design of CY 4031 in order to diminish the potential for the occurrence of dizziness and other AEs known to occur during treatment with *tirasemtiv* to unmask the double-blind treatment assignment. Open-label treatment at study onset also will serve to eliminate those patients unable to tolerate a dose of 125 mg of *tirasemtiv* twice daily prior to randomization and inclusion in the intent to treat cohort.

Treatment with *tirasemtiv* has been associated with a dose-related increase in reports of dizziness in both healthy volunteers and patients with ALS. The dizziness is most often mild and usually resolves with continued dosing. For example, the Phase 2a study, CY 4024, 49 patients with ALS received two weeks' treatment with once daily doses of placebo (n = 13) or *tirasemtiv* at 125 mg, 250 mg, or 375 mg (n = 12 for each group). Dizziness was not reported on placebo in CY 4024 but occurred in 25%, 42%, and 50% of patients receiving *tirasemtiv* at 125 mg, 250 mg, and 375 mg once daily, respectively. As shown below in Figure 6, among patients who reported dizziness, the symptom usually began early after the initiation of treatment and had mostly resolved by the second week.

Figure 6: Incidence of Dizziness by Double-Blind Treatment Assignment and Day of Double-Blind Treatment in CY 4024



In another Phase 2a study, CY 4025, 21 patients with ALS were randomized to receive double-blind *tirasemtiv* administered as 125 mg twice daily for one week, followed by 125 mg in the morning and 250 mg in the evening during the second week, and 250 mg twice daily during the third and final week of treatment. Another six patients were randomized to a dummy dose titration with matching double-blind placebo. None of the patients who received placebo in CY 4025 reported dizziness, while dizziness was reported by 12 of the 21 patients (57%) randomized to dose titration with active *tirasemtiv*. Once again, among the 12 patients who reported dizziness, the symptom usually began early after the initiation of treatment. In 10 of these 21 patients (48%), dizziness began during the first week of *tirasemtiv* treatment at

125 mg BID. Of the 11 patients who did not experience dizziness during that first week of treatment, only two experienced its first onset during the second week, after up-titration to 125 mg in the morning and 250 mg in the evening, and no patients in CY 4025 first experienced dizziness after titration to the highest dose of 250 mg twice daily. Furthermore, in four of the 12 patients who reported dizziness at any time during CY 4025, the symptom resolved during continued administration of *tirasemtiv*, including up-titration to 250 mg twice daily and successful completion of the study in three patients.

In summary, in CY 4024 and CY 4025, roughly half the ALS patients treated with *tirasemtiv* at daily doses of 250 mg and above reported dizziness, which generally began within the first few days of initiating treatment. In many of these patients, however, dizziness tended to resolve with continued dosing, usually within a few days of its first occurrence.

Due to concerns that the occurrence of dizziness and other AEs could contribute to the effective unblinding of patients in BENEFIT-ALS, all patients received one week of open-label treatment with *tirasemtiv* 125 mg BID prior to randomization to double-blind active *tirasemtiv* versus placebo in an effort to diminish this potential for such unmasking of the double-blind treatment assignment. Roughly half the patients enrolled were expected to experience dizziness during open-label treatment with *tirasemtiv* 125 mg BID; however, if dizziness resolved after randomization to double-blind therapy, it could have been due to spontaneous resolution of the symptom despite continued treatment with active *tirasemtiv* (as described above) or due to withdrawal of active *tirasemtiv* and initiation of double-blind placebo.

During BENEFIT-ALS, 291 of the 711 patients enrolled into the open-label portion of the study had at least one treatment-emergent adverse event (TEAE) of dizziness. In these patients, the mean ± standard deviation (SD) time to onset of the first episode of dizziness was 1.1 ± 1.45 days (with a range of 0 to 10.4 days) and the mean ± SD duration of the first episode of dizziness was 14.7 ± 23.97 days (with a range of 0 to 121.4 days). Among these 291 patients, 222 went on to be randomized to placebo or *tirasemtiv*. Among the 111 of these 222 patients randomized to placebo, the mean ± SD duration of the first episode of dizziness that began during the open-label treatment was 14.2 ± 25.47 days (with a range of 0 to 121.4 days). Among the 111 of these 222 patients randomized to *tirasemtiv*, the mean ± SD duration of the first episode of dizziness that began during the open-label treatment was 20.0 ± 27.70 days (with a range of 0 to 113.6 days). There is sufficient similarity between these durations and their ranges to suggest that the week of open-label treatment with *tirasemtiv* prior to randomization was generally effective in preventing dizziness on *tirasemtiv* from unmasking the double-blind treatment assignment of an individual patient. Consequently, an open-label phase prior to randomization has been retained in the design of the current study, CY 4031.

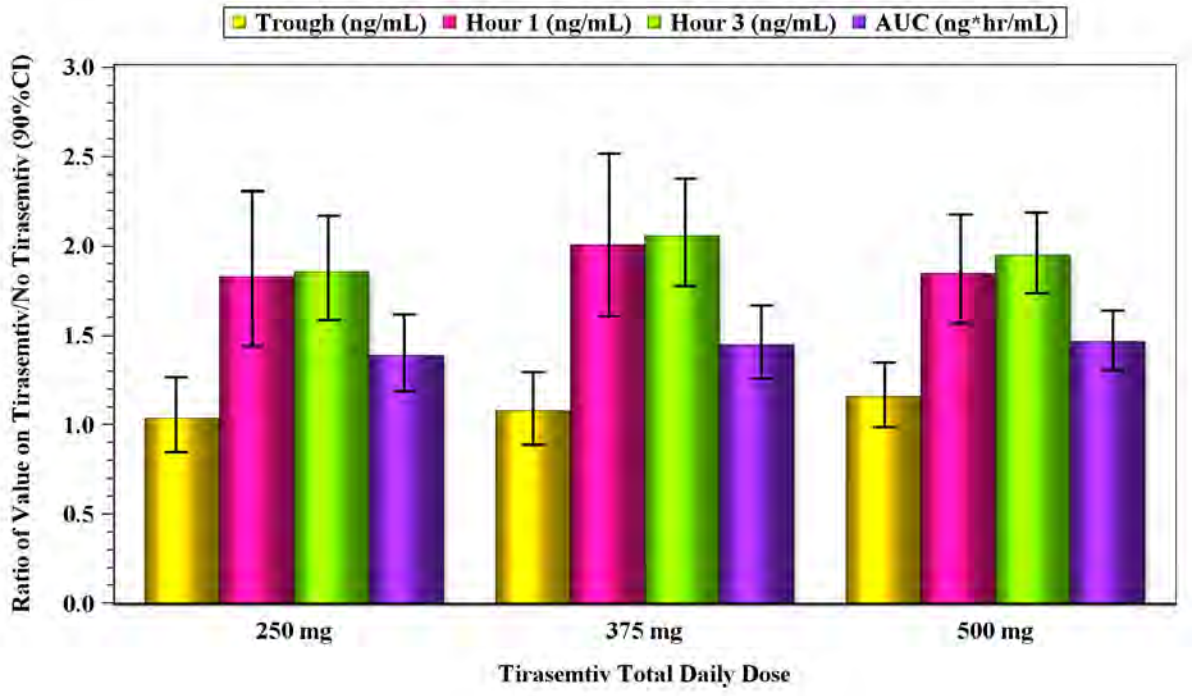
Another purpose of open-label treatment with *tirasemtiv* prior to randomization is to prevent randomization of patients who do not tolerate the starting dose of 125 mg BID. In BENEFIT-ALS, 16% of patients withdrew from open-label treatment prior to randomization; however, 4.7% of patients randomized to *tirasemtiv* withdrew during the first week of double-blind treatment (compared to 1.7% of patients randomized to placebo), even though the *tirasemtiv* dose during that first week was identical to the open-label *tirasemtiv* dose regimen of 125 mg BID. Consequently, the duration of the open-label phase in CY 4031 has been increased to two weeks.

3.4. Riluzole Dose Adjustment Rationale

Tirasemtiv is a mechanism-based inhibitor of the drug metabolizing enzyme CYP1A2, the major pathway of elimination for riluzole; consequently, when *tirasemtiv* is administered concomitantly with riluzole, plasma riluzole concentrations are increased relative to when riluzole is administered in the absence of *tirasemtiv*. Consequently, in BENEFIT-ALS, patients taking riluzole who were randomized to double-blind *tirasemtiv* had their riluzole dose decreased from 50 mg twice daily to 50 mg once daily in a double-blind manner as will be done in the current study, CY 4031 (described in Section 5.8 Concomitant Medications).

With the riluzole dose adjustment employed in BENEFIT-ALS, the analysis depicted in Figure 7, below, showed that during treatment with *tirasemtiv* over the dose range to be studied in CY 4031 (i.e., 125 mg twice daily to 250 mg twice daily), steady-state plasma riluzole concentrations at trough were not increased, while plasma riluzole concentrations at one and three hours after dosing were increased by somewhat less than 2-fold, and riluzole AUC_{24h} values were increased approximately 1.5-fold relative to when riluzole was dosed in the absence of *tirasemtiv*. Importantly, these steady-state riluzole exposures did not vary significantly with the total daily dose (TDD) of *tirasemtiv* over the range from 250 mg to 500 mg.

Figure 7: Steady-State Plasma Riluzole Exposures with and without Steady-State Concomitant *Tirasemtiv* at Total Daily Doses from 250 mg to 500 mg in BENEFIT-ALS



The riluzole dose reduction employed in patients randomized to treatment with double-blind *tirasemtiv* in BENEFIT-ALS did not completely adjust for the effect of *tirasemtiv* to increase plasma riluzole concentrations when the two drugs were administered concomitantly. As shown above, patients randomized to *tirasemtiv* experienced somewhat higher riluzole exposures than patients randomized to placebo, despite the dose reduction. Nevertheless, the riluzole dose

adjustment employed in BENEFIT-ALS will be implemented again in the current study, CY 4031, for the following reasons:

1. Because riluzole is supplied only as 50 mg tablets, it is the only practicable dose adjustment for patients randomized to double-blind *tirasemtiv* that preserves trough riluzole levels in the ranges observed in patients taking riluzole and double-blind placebo in BENEFIT-ALS. Given the demonstrated benefits of riluzole in the treatment of patients with ALS, it is important to err on the side of over-exposure rather than under-exposure.
2. There were no differences in safety or tolerability observed between patients taking riluzole and those not taking riluzole in BENEFIT-ALS. Differences in AE rates between the *tirasemtiv* and placebo treatment groups were generally similar between patients taking riluzole and those not taking riluzole; furthermore, liver function tests (LFT) were similar in patients taking riluzole and those not taking riluzole.

Accordingly, patients who enter CY 4031 taking riluzole will take half of the standard riluzole dose (i.e., 50 mg once daily) during the open-label phase and half of the standard riluzole dose (i.e., 50 mg once daily) during the treatment phases when they are randomized to receive double-blind *tirasemtiv*. Patients who enter CY 4031 taking riluzole and who are randomized to receive double-blind placebo will continue to receive the standard dose of 50 mg twice daily. As noted elsewhere in this protocol (see Section 5.8), the evening riluzole dose will be supplied by the study site during the placebo-controlled phases of the study in a double-blind fashion (active versus placebo riluzole) to preserve blinding of the randomization to *tirasemtiv* versus placebo. In addition, because riluzole exposure is frequently associated with elevations of LFT, LFT will be obtained after initiation of study drug and during dose escalation in CY 4031.

3.5. Double-Blind, Placebo-Controlled, *Tirasemtiv* Withdrawal Phase Rationale

The double-blind, placebo-controlled, *tirasemtiv* withdrawal phase has been incorporated into the design of CY 4031 in order to evaluate the possibility of a "rebound effect" after 48 weeks of treatment with *tirasemtiv*; i.e., increasing symptoms or loss of function after discontinuation of treatment to a point worse than if the patient had not received *tirasemtiv*. This will be evaluated by comparing measures in those patients randomized to *tirasemtiv* for the 48 weeks of double-blind, placebo-controlled treatment but who were then randomized to placebo for the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase to those randomized to placebo throughout the trial during clinic visits scheduled at Week 49 and Week 52. At Week 49, clearance of *tirasemtiv* should have essentially just completed among those patients who had received 48 weeks of *tirasemtiv*, which is when rebound effects might be expected to be at their most prominent, should they occur. The visit at Week 52 will allow assessment of the duration of such rebound effects (if any), or may document their resolution (again, should any such rebound effects be observed).

Of note, there was no evidence for rebound worsening of symptoms or function after discontinuation of *tirasemtiv* following 12 weeks of treatment in the Phase 2b study, BENEFIT-ALS. The assessment for rebound effects in CY 4031, however, will occur after 48 weeks of treatment with active *tirasemtiv*. The substantially longer duration of *tirasemtiv* treatment in

CY 4031 versus BENEFIT-ALS supports the inclusion of an assessment of rebound effects in CY 4031.

4. STUDY POPULATION

Approximately 600 patients who fulfill the eligibility criteria will be enrolled in the study and approximately 477 are expected to be randomized in the double-blind, placebo-controlled phase.

4.1. Inclusion Criteria

1. Able to comprehend and willing to sign an Informed Consent Form (ICF)
2. Male or female 18 years of age or older
3. A diagnosis of familial or sporadic ALS (defined as meeting the possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of ALS according to the World Federation of Neurology El Escorial criteria) ≤ 24 months prior to screening
4. Upright SVC ≥ 70 % of predicted for age, height and sex
5. Able to swallow tablets without crushing, and in the opinion of the Investigator, is expected to continue to be able to do so during the trial
6. A caregiver if one is needed
7. Clinical laboratory findings within the normal range or, if outside the normal range, deemed not clinically significant by the Investigator
8. Male patients must agree for the duration of the study and 10 weeks after the end of the study to use a condom during sexual intercourse with female partners who are of childbearing potential (i.e., following menarche until post-menopausal if not anatomically and physiologically incapable of becoming pregnant) and to have female partners use an additional effective means of contraception (e.g., diaphragm plus spermicide, or oral contraceptives) or the male patient must agree to abstain from sexual intercourse during and for 10 weeks after the end of the study, unless the male patient has had a vasectomy and confirmed sperm count is zero
9. Female patients must be post-menopausal (≥ 1 year) or sterilized, or, if of childbearing potential, not be breastfeeding, have a negative pregnancy test, have no intention to become pregnant during the course of the study, and use effective contraceptive drugs or devices while requiring male partner to use a condom for the duration of the study and for 10 weeks after the end of the study
10. Patients must be either on a stable dose of riluzole 50 mg twice daily for at least 30 days prior to screening or have not taken riluzole for at least 30 days prior to screening and are willing not to begin riluzole use until they complete study drug dosing

4.2. Exclusion Criteria

1. At the time of screening, any use of non-invasive positive pressure ventilation (NIPPV, e.g. continuous positive airway pressure [CPAP] or bi-level positive airway pressure [BiPAP]) for any portion of the day, or mechanical ventilation via tracheostomy, or on any form of oxygen supplementation
2. Patients with a diaphragm pacing system (DPS) at study entry or who anticipate DPS placement during the course of the study

3. BMI of 20.0 kg/m^2 or lower
4. Unwilling or unable to discontinue tizanidine and theophylline-containing medications during study participation
5. Serum chloride outside the normal reference range
6. Neurological impairment due to a condition other than ALS, including history of transient ischemic attack within the past year
7. Presence at screening of any medically significant cardiac, pulmonary, GI, musculoskeletal, or psychiatric illness that might interfere with the patient's ability to comply with study procedures or that might confound the interpretation of clinical safety or efficacy data, including, but not limited to:
    a. Poorly controlled hypertension
    b. NYHA Class II or greater congestive heart failure
    c. Chronic obstructive pulmonary disease or asthma requiring daily use bronchodilator medications
    d. GI disorder that might impair absorption of study drug
    e. History of significant liver disease defined by bilirubin > 2 times the upper limit of normal (ULN) or ALT or AST > 3 times the ULN on repeat testing
    f. Poorly controlled diabetes mellitus
    g. History of vertigo within three months of study entry
    h. History of syncope without an explainable or treated cause
    i. History of untreated intracranial aneurysm or poorly controlled seizure disorder
    j. Amputation of a limb
    k. Cognitive impairment, related to ALS or otherwise, sufficient to impair the patient's ability to give informed consent and to understand and/or comply with study procedures
    l. Cancer with metastatic potential (other than basal cell carcinoma, carcinoma in situ of the cervix, or squamous cell carcinoma of the skin excised with clean margins) diagnosed and treated within the last two years
    m. Any other condition, impairment or social circumstance that, in the opinion of the Investigator, would render the patient not suitable to participate in the study
    n. Patient judged to be actively suicidal or a suicide risk by the Investigator
8. Has taken any investigational study drug within 30 days or five half-lives of the prior agent, whichever is greater, prior to dosing
9. Prior participation in any form of stem cell therapy for the treatment of ALS
10. Previously received *tirasemtiv* in any previous clinical trial

4.3. Additional Randomization Criterion

Patients must tolerate two weeks of open-label treatment with *tirasemtiv* 125 mg twice daily to be eligible for randomization.

5. STUDY PROCEDURES

5.1. Screening

A signed ICF will be obtained prior to any screening procedures and before any study specific assessments are initiated.

The following screening procedures will be performed for all potential patients at a visit conducted within 14 days of start of the open-label phase of the study:

1. Informed consent documentation
2. Inclusion/exclusion criteria evaluation
3. Demographic data collection
4. Medical history collection (includes smoking history)
5. Clinical safety laboratory evaluations including a serum chemistry panel, complete blood count (CBC), urinalysis (UA), creatine phosphokinase (CPK), and thyroid stimulating hormone (TSH)
6. Serum pregnancy test for females of child bearing potential
7. Vital signs measurements (sitting for at least three minutes) including blood pressure, pulse, respiration rate and temperature
8. 12-lead electrocardiogram (ECG)
9. Routine physical examination, including height and weight
10. Neurological examination
11. ALSFRS-R
12. Respiratory assessment
13. Concomitant medication assessment
14. Ashworth score
15. Suicidality assessment

Confirmation of eligibility for enrollment into the open-label phase must occur prior to the Week -2 visit.

5.2. Open-Label Phase

5.2.1. WEEK -2 VISIT

Week -2 is the first study visit after screening and the start of the open-label phase. Patients will report to the study site for the Week -2 visit which may occur at any time up to, but not after 14 days following the Screening Visit. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of riluzole (for patients on riluzole) prior to arrival at the study site.

The following procedures will then be performed:

1. PK blood sampling
2. Clinical Safety laboratory evaluations including serum chemistry panel, CBC, UA, and CPK
3. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight
4. Respiratory assessment
5. Handgrip strength (bilateral)
6. Muscle strength measurements
7. ALSFRS-R
8. ALS Assessment Questionnaire Short Form (ALSAQ-5)
9. Epworth Sleepiness Scale
10. Falls assessment
11. Caregiver Burden assessment
12. AE evaluation and concomitant medication assessment (since last visit)
13. Suicidality assessment

Once the assessments are completed at the Week -2 visit, patients will receive their first morning dose of open-label study drug (125 mg of *tirasemtiv*). Patients currently taking riluzole also will take 50 mg riluzole from their personal supply.

Patients will be given an adequate supply of open-label study drug (*tirasemtiv*) to take twice daily for 14 days at home until their next clinic visit (Day 1 Visit). A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and <u>not</u> to take their evening dose of riluzole during the open-label phase. Patients will be contacted by phone after seven days of open-label *tirasemtiv* and will return to the study site after 14 days of open-label treatment for the Day 1 visit.

5.3. Double-Blind, Placebo-Controlled Phase

The double-blind, placebo-controlled phase will last approximately 48 weeks. Patients who have demonstrated adequate tolerance of *tirasemtiv* during the open-label phase will be randomized to *tirasemtiv* versus placebo prior to the Day 1 procedures. Randomization should occur following completion of the open-label phase and prior to Day 1 visit procedures. Randomization must not occur until the patient has been contacted by study site personnel at the end of the open-label phase and the Investigator is confident that the patient has tolerated the open-label phase such that, if any adverse symptoms that the patient experienced during open-label treatment were to continue throughout the double-blind portion of the study with the same severity, those symptoms would be sufficiently mild that they would not likely prevent the patient from

completing the study. The patient, caregiver, and the Investigator will all be blinded to the patient's treatment group assignment.

5.3.1. DAY 1 VISIT

Day 1 marks the beginning of the double-blind, placebo-controlled phase. During the first two weeks of the double-blind, placebo-controlled phase, all patients will take a TDD of 250 mg of *tirasemtiv* or placebo for 14 days.

Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Biomarker sample
3. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
4. Respiratory assessment
5. ALSFRS-R
6. Falls assessment
7. AE evaluation and concomitant medication assessment (since last visit)
8. Suicidality assessment

Once all pre-dose procedures have been performed at the Day 1 visit, patients will take their morning dose of assigned study drug (125 mg of *tirasemtiv* or placebo). For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will begin double-blind dosing as follows for the next two weeks.

Patients randomized to receive double-blind *tirasemtiv*:

- Patients randomized to the TDD of 250 mg, 375 mg or 500 mg of *tirasemtiv* will begin dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for two weeks

Patients randomized to receive double-blind placebo will begin dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for two weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for two weeks at home until their next clinic visit (Week 2 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole). Patients on riluzole

will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

5.3.2. WEEK 2 VISIT

Patients will return approximately two weeks later to the study site for the Week 2 visit. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of riluzole and study drug while in the clinic:

1. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
2. Falls assessment
3. AE evaluation and concomitant medication assessment (since last visit)
4. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 2 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next two weeks.

Patients randomized to receive double-blind *tirasemtiv*:

- For patients randomized to the TDD of 250 mg of *tirasemtiv*, patients will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for two weeks
- For patients randomized to the TDD of 375 mg or 500 mg of *tirasemtiv*, patients will begin dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for two weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for two weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for two weeks at home until their next clinic visit (Week 4 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

5.3.3. WEEK 4 VISIT

Patients will return approximately two weeks later to the study site for the Week 4 visit and continue with dosing. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Clinical Safety laboratory evaluations including serum chemistry panel, CBC, UA, and CPK
3. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
4. Respiratory assessment
5. Handgrip strength (bilateral)
6. Muscle strength measurements
7. ALSFRS-R
8. Ashworth score
9. Falls assessment
10. AE evaluation and concomitant medication assessment (since last visit)
11. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 4 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next four weeks.

Patients randomized to receive double-blind *tirasemtiv:*

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for four weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will begin dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for four weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for four weeks at home until their next clinic visit (Week 8 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

5.3.4. WEEK 8 VISIT

Patients will return approximately four weeks later to the study site for the Week 8 visit and continue with dosing. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of morning dose of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Biomarker sample
3. Clinical Safety laboratory evaluations including serum chemistry panel, CBC, UA, and CPK
4. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
5. 12-lead ECG
6. Respiratory assessment
7. Handgrip strength (bilateral)
8. Muscle strength measurements
9. ALSFRS-R
10. Ashworth score
11. Falls assessment
12. AE evaluation and concomitant medication assessment (since last visit)
13. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 8 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next four weeks.

Patients randomized to receive double-blind *tirasemtiv:*

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for four weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will continue dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for four weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for four weeks at home until their next clinic visit (Week 12 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

5.3.5. WEEK 12 VISIT

Patients will return approximately four weeks later to the study site for the Week 12 visit and continue with dosing. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of morning dose of riluzole and study drug while in the clinic:

1. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
2. Respiratory assessment
3. Handgrip strength (bilateral)
4. Muscle strength measurements
5. ALSFRS-R
6. ALSAQ-5
7. Epworth Sleepiness Scale
8. Ashworth score

9. Falls assessment
10. AE evaluation and concomitant medication assessment (since last visit)
11. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 12 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next four weeks.

Patients randomized to receive double-blind *tirasemtiv:*

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for four weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will continue dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for four weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for four weeks at home until their next clinic visit (Week 16 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

5.3.6. WEEK 16 VISIT

Patients will return approximately four weeks later to the study site for the Week 16 visit and continue with dosing. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of morning dose of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Biomarker sample

3. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
4. Respiratory assessment
5. Handgrip strength (bilateral)
6. Muscle strength measurements
7. ALSFRS-R
8. Falls assessment
9. AE evaluation and concomitant medication assessment (since last visit)
10. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 16 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next four weeks.

Patients randomized to receive double-blind *tirasemtiv:*

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for four weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will continue dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for four weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for four weeks at home until their next clinic visit (Week 20 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

5.3.7. WEEK 20 VISIT

Patients will return approximately four weeks later to the study site for the Week 20 visit and to continue with dosing. Patients will be instructed to bring their own riluzole supply to the study

visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of morning dose of riluzole and study drug while in the clinic:

1. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
2. Respiratory assessment
3. Handgrip strength (bilateral)
4. Muscle strength measurements
5. ALSFRS-R
6. Falls assessment
7. AE evaluation and concomitant medication assessment (since last visit)
8. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 20 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next four weeks.

Patients randomized to receive double-blind *tirasemtiv:*

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for four weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will continue dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for four weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for four weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for four weeks at home until their next clinic visit (Week 24 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole). Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their

morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study visit.

5.3.8. WEEK 24 VISIT

The end of Week 24 marks the halfway point in the double-blind, placebo-controlled phase.

Patients will return approximately four weeks later to the study site for the Week 24 visit and to continue with dosing. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of morning dose of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Biomarker sample
3. Clinical Safety laboratory evaluations including serum chemistry panel, CBC, UA, and CPK
4. Serum pregnancy test for females of child bearing potential
5. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
6. 12-lead ECG
7. Respiratory assessment
8. Handgrip strength (bilateral)
9. Muscle strength measurements
10. Routine physical examination
11. Neurological examination
12. ALSFRS-R
13. ALSAQ-5
14. Epworth Sleepiness Scale
15. Ashworth score
16. Falls assessment
17. Caregiver burden assessment
18. AE evaluation and concomitant medication assessment (since last visit)
19. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 24 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next eight weeks.

Patients randomized to receive double-blind *tirasemtiv:*

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for eight weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for eight weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will continue dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for eight weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for eight weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for eight weeks at home until their next clinic visit (Week 32 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study visit.

5.3.9. WEEK 32 VISIT

Patients will return approximately eight weeks later to the study site for the Week 32 visit. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of morning dose of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Biomarker sample
3. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
4. Respiratory assessment
5. Handgrip strength (bilateral)
6. Muscle strength measurements
7. ALSFRS-R

8. Falls assessment
9. AE evaluation and concomitant medication assessment (since last visit)
10. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 32 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next eight weeks.

Patients randomized to receive double-blind *tirasemtiv*:

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for eight weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for eight weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will continue dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for eight weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for eight weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for eight weeks at home until their next clinic visit (Week 40 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

5.3.10. WEEK 40 VISIT

Patients will return approximately eight weeks later to the study site for the Week 40 visit. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of morning dose of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Biomarker sample

3. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
4. Respiratory assessment
5. Handgrip strength (bilateral)
6. Muscle strength measurements
7. ALSFRS-R
8. Falls assessment
9. AE evaluation and concomitant medication assessment (since last visit)
10. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 40 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as follows for the next eight weeks.

Patients randomized to receive double-blind *tirasemtiv*:

- Patients randomized to the TDD of 250 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the evening for eight weeks
- Patients randomized to the TDD of 375 mg of *tirasemtiv* will continue dosing with 1 tablet (125 mg) of *tirasemtiv* and 1 tablet of matching placebo in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for eight weeks
- Patients randomized to the TDD of 500 mg of *tirasemtiv* will continue dosing with 2 tablets (250 mg) of *tirasemtiv* in the morning and 2 tablets (250 mg) of *tirasemtiv* in the evening for eight weeks

Patients randomized to receive double-blind placebo will continue dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for eight weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for eight weeks at home until their next clinic visit (Week 48 visit). Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)*. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

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

The double-blind, placebo-controlled, *tirasemtiv* withdrawal phase will last approximately four weeks. Upon completion of the double-blind, placebo-controlled phase, patients will be

randomized to continue active dosing or to withdraw from active treatment and receive placebo for the next four weeks.

Randomization should occur following completion of the double-blind, placebo-controlled phase and prior to Week 48 visit procedures.

5.4.1. WEEK 48 VISIT

The Week 48 visit marks the beginning of the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of the morning dose of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Biomarker sample
3. Clinical Safety laboratory evaluations including serum chemistry panel, CBC, UA, and CPK
4. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
5. 12-lead ECG
6. Respiratory assessment
7. Handgrip strength (bilateral)
8. Muscle strength measurements
9. Routine physical examination
10. Neurological examination
11. ALSFRS-R
12. ALSAQ-5
13. Epworth Sleepiness Scale
14. Ashworth score
15. Falls assessment
16. Caregiver burden assessment
17. AE evaluation and concomitant medication assessment (since last visit)
18. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 48 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing for the next four weeks based on the second randomization as follows (refer to Figure 4):

- **Patients previously on double-blind *tirasemtiv* and randomized to double-blind *tirasemtiv*** will maintain the *tirasemtiv* dose previously taken during the double-blind, placebo-controlled phase for the next four weeks.
- **Patients previously on double-blind *tirasemtiv* and randomized to double-blind placebo** will begin dosing with 2 placebo tablets in the morning and 2 placebo tablets in the evening for the next four weeks.
- **Patients previously on double-blind placebo will be randomized to continue to receive double-blind placebo** with 2 placebo tablets in the morning and 2 placebo tablets in the evening for the next four weeks.

Patients will be given an adequate supply of study drug (*tirasemtiv* or placebo) to take twice daily for four weeks at home. Patients taking riluzole will also be given a supply of double-blind riluzole or placebo riluzole to take as the evening riluzole dose at home. A diary will be provided for patients to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole). Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study drug.

Patients will be reminded that even though they will receive a four week supply of study drug at this visit, they will need to return for a study visit (Week 49) following one week of treatment in the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase.

5.4.2. WEEK 49 VISIT

Patients will return approximately one week later to the study site for the Week 49 visit. Patients will be instructed to bring their own riluzole supply to the study visit and not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed prior to administration of the morning dose of riluzole and study drug while in the clinic:

1. PK blood sampling
2. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
3. Respiratory assessment
4. Handgrip strength (bilateral)
5. Muscle strength measurements
6. ALSFRS-R
7. Falls assessment
8. AE evaluation and concomitant medication assessment (since last visit)

9. Suicidality assessment

Once all pre-dose procedures have been performed at the Week 49 visit, patients will take their morning dose of assigned study drug. For patients taking riluzole, 50 mg riluzole from their personal supply should also be taken with the morning dose of study drug.

Patients will continue study drug dosing as described in the Week 48 visit for the remaining three weeks of the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase. Patients should be reminded to continue to record the date and time of twice daily study drug administration and riluzole administration (if on riluzole*)* in their patient diary. Patients on riluzole will be reminded to take 50 mg riluzole from their personal supply at the same time as their morning dose of study drug and riluzole from the provided supply of double-blind riluzole (active or placebo) at the same time as their evening dose of study assignment.

5.4.3. WEEK 52 VISIT

Patients will return approximately three weeks later to the study site for the Week 52 visit. Patients will be instructed not to take their morning dose of study drug or riluzole (for patients on riluzole) prior to arrival at the study site.

The following assessments will be performed while in the clinic:

1. PK blood sampling
2. Clinical Safety laboratory evaluations including serum chemistry panel, CBC, UA, and CPK
3. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
4. 12-lead ECG
5. Respiratory assessment
6. Maximum handgrip (bilateral)
7. Muscle strength measurements
8. ALSFRS-R
9. Falls assessment
10. AE evaluation and concomitant medication assessment (since last visit)
11. Suicidality assessment

The Week 52 visit represents the end of dosing. Patients on riluzole will resume taking their prescribed 50 mg riluzole dose from their personal supply starting the evening of the Week 52 visit, and their prescribed 50 mg riluzole twice daily starting the day after the Week 52 visit.

5.5. FOLLOW-UP VISIT (Week 56)

Patients will return to the study site approximately four weeks following the last dose of study drug taken during the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase, and the following final assessments will be performed:

1. Clinical Safety laboratory evaluations including serum chemistry panel, CBC, UA, and CPK
2. Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight (if the patient has lost ≥ 5% of body weight since baseline, the Investigator will be alerted)
3. 12-lead ECG
4. Respiratory assessment
5. Maximum handgrip (bilateral)
6. Muscle strength measurements
7. Routine physical examination
8. Neurological examination
9. ALSFRS-R
10. ALSAQ-5
11. Epworth Sleepiness Scale
12. Ashworth score
13. Falls assessment
14. Caregiver burden assessment
15. AE evaluation and concomitant medication assessment (since last visit)
16. Suicidality assessment

Upon collection of the assessments listed above, patients will have completed their participation in the study.

5.6. Visit Windows

To aid in scheduling patient visits, the following study visit windows are considered acceptable (Table 8). If a patient visit must be scheduled outside the visit window, the Medical Monitor should be contacted.

Table 8: Visit Windows

Visit	Visit Window
Week -4	Screening up to 14 days prior to Week -2 Visit (as per protocol)
Week -2	14 days prior to Day 1 Visit, + **2 days**
Day 1	First day of randomized, double-blind dosing
Week 2	+/- **2 days**
Week 4	+/- **2 days**
Week 8	+/- **4 days**
Week 12	+/- **4 days**
Week 16	+/- **4 days**
Week 20	+/- **4 days**
Week 24	+/- **4 days**
Week 32	+/- **7 days**
Week 40	+/- **7 days**
Week 48	+/- **7 days**
Week 49	+/- **1 day**
Week 52	+/- **3 days**
Week 56	Follow-Up 4 weeks after last dose, +/- **3 days**

5.7. Timing of Doses

Study drug (*tirasemtiv* or placebo) should be taken in the morning at home (except on study visit days where it should be taken in the clinic) and in the evening, approximately 12 hours apart. *Tirasemtiv* should be taken ≥ 2 hours after a meal or 1 hour prior to a meal. For patients taking riluzole, *tirasemtiv* may be taken at the same time as riluzole.

5.8. Concomitant Medications

All prescription drugs, over-the-counter medications, nutriceuticals and herbal remedies taken by the patient from the time of the screening visit through the follow-up visit should be entered into the electronic case report form (eCRF).

Patients who were intolerant to riluzole or chose not to take riluzole prior to study entry, will be encouraged not to take riluzole until they complete study drug dosing. Patients who were not taking riluzole at study entry but who later decide to start riluzole during the dosing phase of the study will not be allowed to continue taking study drug. When patients are taking *tirasemtiv* during the study, their dose of riluzole will be reduced to 50 mg once daily in the morning as described below.

In order to preserve the blind with respect to assignment to *tirasemtiv* versus placebo, patients taking riluzole will take 50 mg riluzole from their personal supply in the morning and during the placebo-controlled phases of the study (double-blind, placebo-controlled phase and the double-blind, placebo-controlled *tirasemtiv* withdrawal phase), a blinded (active or placebo) riluzole

tablet (supplied by the study site) in the evening. Patients will resume taking their prescribed 50 mg riluzole twice daily from their personal supply starting at Week 52.

Thus, patients taking riluzole prior to study entry will have their riluzole adjusted, as follows:

- During the open-label phase, patients will take 50 mg riluzole from their personal supply in the morning and will not take an evening riluzole dose.
- During the double-blind, placebo-controlled phase (Day 1 – Week 48), patients will take their morning dose of riluzole (50 mg once daily) and will take a blinded (active or placebo) riluzole tablet (supplied by the study site) in the evening.
- During the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase (Week 48 – Week 52), patients will take their morning dose of riluzole (50 mg once daily) and will take a blinded (active or placebo) riluzole tablet (supplied by the study site) in the evening.
- Patients will resume taking their prescribed 50 mg riluzole twice daily from their personal supply starting at Week 52.

Tirasemtiv inhibits multiple CYP450 isoforms (1A2, 2B6, 2C8, 2C9, and 2C19). Consequently, exposure to drugs primarily metabolized by these isoforms may be increased when these drugs are given in conjunction with *tirasemtiv*. Therefore, close attention should be paid to potential AEs related to their administration. For example, blood pressure should be closely monitored for hypertensive patients taking angiotensin II receptor blockers metabolized by CYP 2C9, such as losartan and irbesartan. Diabetic patients taking oral anti-diabetic agents metabolized by CYP 2C8 or 2C9, including glyburide, glibencilamide, glipizide, glimepride and tolbutamide, should be cautioned to be aware of signs and symptoms of hypoglycemia, and serum glucose should be monitored as clinically appropriate. Finally, caution should be exercised in the initiation of warfarin (also metabolized by CYP 2C9) if required during the study for the treatment of thromboses or thromboembolic events, with careful monitoring of prothrombin times and/or international normalized ratio (INR). A list of currently approved drugs metabolized by CYP 2B6, 2C8, 2C9, and 2C19 may be found in Appendix B.

Patients must refrain from taking theophylline or tizanidine, which are primarily metabolized by CYP 1A2 (see Section 1.3), during their participation in the study. Additionally, patients should be advised to avoid or use caution when taking medications or ingesting other substances that are metabolized by CYP 1A2, such as fluvoxamine, because their metabolism is inhibited by *tirasemtiv*, so co-administration with *tirasemtiv* will increase exposure to these compounds.

Other medications that inhibit or induce the activity of CYP 1A2 may increase or decrease, respectively, exposure to riluzole which is metabolized by CYP 1A2. Consequently, these drugs should be avoided by patients taking riluzole. A list of currently approved drugs that interact with CYP 1A2 is found in Appendix C.

5.9. PK Sample Collection

Blood samples for PK analysis of *tirasemtiv*, and its acid metabolite, CK-2018595, and riluzole (if the patient is taking riluzole) will be collected at the time points listed in Table 9. The morning study drug and riluzole dose should be taken at the site on these days. Patients will be

instructed NOT to take their morning dose of study drug or their dose of riluzole until after the pre-dose PK blood draw.

Table 9: Pharmacokinetic (PK) Samples

Visit	Sample Time Points
Week -2	Pre-AM dose
Day 1	Pre-AM dose
Week 4	Pre-AM dose
Week 8	Pre-AM dose
Week 16	Pre-AM dose
Week 24	Pre-AM dose
Week 32	Pre-AM dose
Week 40	Pre-AM dose
Week 48	Pre-AM dose
Week 49	Pre-AM dose
Week 52	Pre-AM dose

PK plasma samples remaining after completion of bioanalysis may also be used for exploratory analysis of *tirasemtiv* metabolites. These samples will NOT be used for pharmacogenomic or other testing.

5.10. Biomarker Sample Collection

Biomarkers are objective measures or indicators of normal biological processes, pathological processes or pharmacological responses to a therapeutic intervention. Biomarker development may be useful in developing markers to identify disease subtypes, to guide therapy, or predict disease severity. Where authorized by the applicable IRB/EC/REB approved informed consent, blood samples for biomarker development will be collected at the time points shown in Table 10.

Table 10: Biomarker Samples

Visit	Sample Time Points
Day 1	Pre-AM dose
Week 8	Pre-AM dose
Week 16	Pre-AM dose
Week 24	Pre-AM dose
Week 32	Pre-AM dose
Week 40	Pre-AM dose
Week 48	Pre-AM dose

Plasma samples will be stored for future biomarker analyses and will NOT be used for pharmacogenomic testing.

5.11. Clinical Safety Assessments

5.11.1. Physical Examination

A routine physical exam will be performed at Screening, Week 24, Week 48, and the Follow-Up Visit.

5.11.2. Neurological Examination

The neurological exam will be administered as described in the study manual and will include an assessment of cranial nerve function (including extra-ocular movements), motor strength, and sensory function.

A neurological exam will be performed at Screening, Week 24, Week 48, and the Follow-Up Visit.

5.11.3. Ashworth Score

The Ashworth Score will be assessed as described in the study manual at Screening, Week 4, Week 8, Week 12, Week 24, Week 48 and the Follow-Up Visit.

5.11.4. Clinical Laboratory Evaluations

Clinical safety laboratory evaluations (serum chemistry panel, CBC, UA, and CPK) will be collected at Screening, Week -2, Week 4, Week 8, Week 24, Week 48, Week 52, and the Follow-Up Visit. A TSH will be collected only at the Screening visit and a serum pregnancy test for females of child bearing potential will be collected at Screening and the Week 24 visit.

5.11.5. 12-Lead ECGs

A 12-lead ECG will be obtained at Screening, Week 8, Week 24, Week 48, Week 52, and the Follow-Up Visit.

5.11.6. Vital Signs

Vital signs measurements (including blood pressure, pulse, respiration rate after sitting for at least three minutes, and temperature) as well as body weight will be recorded at all study visits.

5.11.7. Falls Assessment

A fall is defined as a sudden, unintentional change in position causing an individual to land at a lower level on an object, the floor, the ground, or other surface. For the purposes of this study, the definition of a fall does not include stumbles or trips from which the patient rights him/herself without landing lower than his/her previous position (Tinetti, Baker et al. 2004). A falls assessment will be performed at each clinic visit, starting at Week -2 through the final Follow-Up Visit.

5.11.8. Suicidality Assessment

The suicidality assessment will be assessed as described in the study manual at all study visits.

5.12. Clinical and Pharmacodynamic Outcome Measures

5.12.1. ALS Functional Rating Scale-Revised (ALSFRS-R)

The ALSFRS-R will be administered as described in the study manual at all study visits except Week 2. When a patient does not present in person for a scheduled visit, every attempt should be made to obtain the ALSFRS-R score by telephone as described in the study manual.

5.12.2. Respiratory Assessments

The respiratory assessments in this study include SVC and SNIP. They will be performed as described in the study manual at every study visit except Week 2.

5.12.3. Handgrip Strength

Maximum handgrip strength will be measured bilaterally as described in the study manual at every study visit except Screening, Day 1, and Week 2.

5.12.4. Muscle Strength Measurements

Muscle strength measurements (elbow flexion, wrist extension, knee extension, and ankle dorsiflexion) will be performed using the Hand Held Dynamometer (HHD), as described in the study manual at every study visit except Screening, Day 1, and Week 2.

5.12.5. Quality of Life

The ALS Assessment Questionnaire Short Form (ALSAQ-5) will be used to assess the patient's overall sense of well-being as described in the study manual at Week -2, Week 12, Week 24, Week 48, and the Follow-Up Visit.

5.12.6. Epworth Sleepiness Scale

The Epworth Sleepiness Scale will be assessed as described in the study manual at Week -2, Week 12, Week 24, Week 48 and the Follow-Up Visit.

5.12.7. Caregiver Burden Assessment

Caregiver burden will be assessed as described in the study manual at Week -2, Week 24, Week 48, and the Follow-Up Visit.

5.13. Patient Discontinuation from Study Participation

Patients will be informed that they are free to withdraw from the study at any time and for any reason. The Investigator may remove a patient from the study at any time if, in the Investigator's opinion, it is not in the best interest of either the patient or the study for the patient to continue in the study. For those patients who will not return to the study center for further study scheduled assessments as described below, the Follow-Up Visit (Week 56) study assessments should be performed and recorded. Notification of discontinuation will immediately be made to the Sponsor's Medical Monitor.

In all cases, patients who were randomized and discontinue study drug should be strongly encouraged to perform all remaining study visits and assessments for the duration of the study

and most importantly the assessments at 24 weeks rather than withdraw from the study. Unless patients withdraw their consent for further contact, those patients who are unable to attend scheduled study visits, should be contacted on a monthly basis to obtain vital status and respiratory status (i.e., use of non-invasive ventilation or permanent mechanical ventilation) through 48 weeks.

The date the patient is withdrawn from study drug treatment or study participation and the reason(s) for discontinuation will be recorded on the patient's eCRF. See Section 7.5.1 regarding follow-up of AEs (both non-serious and serious) continuing at the time of study discontinuation.

5.14. Study Discontinuation

An Investigator may discontinue the participation of his/her study site, or the entire study may be discontinued at the discretion of the Sponsor, based on the occurrence of the following:

- AEs
- medical or ethical reasons affecting the continued performance of the study
- difficulties in the recruitment of patients
- cancellation of drug development

5.15. Safety Monitoring and Stopping Rules

Individual AEs meeting any of the following criteria will be reviewed by the head of Drug Safety and the Sponsor's Medical Monitor in a blinded manner on a periodic basis:

1. SAEs thought by the Sponsor or the Investigator to be related to study drug administration
2. Withdrawals due to AEs (both serious and non-serious)
3. Grade 3 or 4 (according to Common Terminology Criteria for Adverse Events [CTCAE version 4.0], see Section 7.2) non-serious AEs thought by the Sponsor to be related to study drug administration
4. Any death thought by the sponsor or the investigator to be related to study drug will result in stopping all randomization in order to perform a review.

This review may result in:

- Discontinuation of the study
- Discontinuation of enrollment at the affected and higher dose levels
- Modification to study conduct, or
- No change to the conduct of the study (if AEs are determined to have been unrelated to study drug administration)

5.16. Data Monitoring Committee

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.

6. INVESTIGATIONAL PRODUCT

6.1. Description of Investigational Product

This is a double-blind, placebo-controlled study. As such, the site pharmacy staff, the Investigator, the patient, and remaining study site clinical staff will be blinded to treatment assignment.

CK-2017357 *(tirasemtiv)* study drug is supplied as immediate release, white, modified oval tablets at a dose strength of 125 mg of *tirasemtiv* per tablet, which are to be stored under secure conditions at controlled room temperature (20-25°C; 68-77°F) with temporary excursions allowed between 15-30°C; 59-86°F. Matching placebo tablets will be supplied and should also be stored at controlled room temperature under secure conditions. Active CK-2017357 tablets contain 125 mg of *tirasemtiv* plus excipients. Placebo tablets contain excipients only.

Table 11: Study Drug

Study Drug	**CK-2017357**	**Placebo for CK-2017357**
Form	Tablet	Tablet
Supplier	Cytokinetics, Inc.	Cytokinetics, Inc.
Manufacturer	Patheon, Inc.	Patheon, Inc.

CK-2017357 *(tirasemtiv)* and matching placebo tablets will be supplied to the clinical site in blister strips inside labeled wallets. During the open-label phase of the study, patients will receive a bottle containing 40 tablets of *tirasemtiv*, sufficient for two weeks of dosing plus overage. Open-label bottles will be labeled with a lot number. During the double-blind randomized phases of the study, patients will receive one or more wallets containing 72 tablets of study drug (*tirasemtiv* or matching placebo) at each visit. Each wallet is sufficient for two weeks of dosing, plus overage. Double-blind wallets will be labeled with a unique random kit number.

The site pharmacist or other qualified person responsible for managing study drug supplies will maintain an accurate record of the receipt of the investigational study drug as shipped by the Sponsor (or designee), including the kit number and date received. One copy of this receipt will be returned to the Sponsor when the contents of the investigational study drug shipment have been verified. The study research coordinator or designated site staff will be responsible for patient randomizations, using an IWRS. In addition, an accurate drug disposition record will be kept, specifying the kit number provided to each patient and the dates of dose administration. This drug accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by the Sponsor upon request.

All unused drug supplies will be returned to the Sponsor (or designee) or disposed of by the study site, per the Sponsor's (or designee's) instructions at the end of the study.

6.1.1. Description of Riluzole or Placebo for Riluzole

This is a double-blind, placebo-controlled study. As such, the site pharmacy staff, the Investigator, the patient, and remaining study site clinical staff will be blinded to riluzole or placebo treatment.

Riluzole and matching placebo will be supplied as white, capsule shaped tablets. One riluzole tablet will contain 50 mg riluzole plus excipients. Placebo tablets for riluzole will contain excipients only. Riluzole and matching placebo tablets are to be stored under secure conditions at controlled room temperature (20-25°C; 68-77°F) with temporary excursions allowed between (15-30°C; 59-86°F).

Table 12: Riluzole and Placebo for Riluzole

Study Drug	**Riluzole**	**Placebo for Riluzole**
Form	Tablet	Tablet
Supplier	Cytokinetics, Inc.	Cytokinetics, Inc.
Manufacturer	Almac Clinical Services, LLC	Almac Clinical Services, LLC

Riluzole and placebo for riluzole will be supplied to the clinical site in blister strips inside labeled wallets. Patients will receive one or more wallets, containing 18 riluzole or placebo for riluzole tablets at each visit. Each wallet is sufficient for two weeks of dosing, plus overage. Double-blind wallets will be labeled with a unique random kit number.

6.2. Dose Administration (*Tirasemtiv* or Placebo)

This study drug will be administered orally as tablets to patients with ALS. Doses (*tirasemtiv* or placebo) for each of the treatment groups will be dispensed in accordance with the study randomization prior to the patient's first dose.

6.3. Dose Adjustment Criteria

Patients who do not tolerate a dose escalation due to symptoms believed to be due to treatment with study drug may be returned to a previously tolerated dose level at the discretion of the Investigator. No upward dose adjustments will be allowed subsequent to a dose reduction.

6.4. Treatment Interruption

Patients who do not take the total assigned daily dose of study drug due to conditions of hospitalization or other circumstances should be encouraged to return to treatment. The Medical Monitor should be contacted for a patient who has discontinued treatment for more than one week. Specific instructions regarding treatment interruption are provided in the study manual.

6.5. Randomization Schedule and Removal of Blind

There are two separate randomizations in this study; one prior to the start of the double-blind, placebo-controlled phase, and one prior to the start of the double-blind, placebo-controlled, *tirasemtiv* withdrawal phase for all patients. An IWRS will provide patient randomization assignments for this study. The site pharmacist or other qualified person responsible for randomizing patients will receive the appropriate kit numbers through the IWRS and provide it to the site staff who will be distributing study drug to the patient. RANDOMIZATION INFORMATION MAY BE MADE AVAILABLE TO THE INVESTIGATOR ONLY IN THE EVENT OF A MEDICAL EMERGENCY OR AN AE THAT NECESSITATES IDENTIFICATION OF THE STUDY DRUG (*tirasemtiv* or placebo) FOR THE WELFARE OF

THAT PATIENT. Except in a medical emergency, the Investigator (or designee) and study site clinical staff will remain blinded during the conduct of the study and until such time that all discrepancies in the clinical database are resolved (i.e., at the time of the database lock). The date/initials and reason for study blind removal by the Investigator and/or clinical staff will be documented. The Investigator will contact the Sponsor as soon as possible before or immediately following the emergency unblinding of any patient.

6.6. Daily Dosing Diary

A dosing diary will be maintained by the patient to record date and time of twice daily study drug administration plus twice daily riluzole (if taking riluzole). The dosing diary should be returned at each clinic visit.

6.7. Study Drug Handling and Disposal

The site pharmacist or other qualified person responsible for managing study drug supplies will maintain an accurate record of the receipt of the investigational study drug as shipped by the Sponsor (or designee), including the kit number and date received. One copy of this receipt will be returned to the Sponsor when the contents of the investigational study drug shipment have been verified. The study research coordinator or designated study staff will be responsible for patient randomization, using an IWRS. In addition, an accurate drug disposition record will be kept, specifying the kit number provided to each patient and the dates of dose administration. This drug accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by the Sponsor upon request.

All unused drug supplies will be returned to the Sponsor (or designee) or disposed of by the study site, per the Sponsor's (or designee's) instructions at the end of the study.

6.8. Study Drug Accountability

The patient should be instructed to return all unused study drug to the designated clinical site staff at each study visit. Study drug accountability is to be conducted by the designated study staff member with the patient at each study visit. The dosing diaries should also be referenced when completing drug accountability.

7. ADVERSE EVENTS AND SAFETY ASSESSMENTS

NOTE: the term "adverse event (AE)" includes both non-serious and serious AEs unless otherwise specified.

The assessment of safety during the course of this study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medication and measurements of protocol-specific physical examination findings and laboratory tests.

7.1. Definitions

7.1.1. Adverse Event

As defined by the International Council on Harmonisation (ICH) E2A guideline Definitions and Standards for Expedited Reporting, and 21 CFR 312.32, Investigational New Drug (IND) Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Consider the following information when determining whether or not to record a test result, medical condition or other incident on the AE eCRF:

- From the time of informed consent to first administration of study drug (Week -2 visit), only study protocol-related AEs should be recorded
- New symptoms or diagnoses reported/found between the time of signing of informed consent and the first administration of study drug will be recorded as medical history on the medical history eCRF
- Conditions newly detected or diagnosed after administration of study drug, including conditions that may have been present but undetected prior to the start of the study should be recorded
- All AEs (regardless of relationship to study drug) should be recorded from first administration of study drug (Week -2 visit) through the end of the safety reporting period (see Section 7.5, Reporting Period for AEs and SAEs)
- In general, an abnormal laboratory value should not be recorded as an AE unless it is assessed by the Investigator as clinically significant (e.g., associated with clinical signs or symptoms, requires intervention, results in a SAE, or results in study termination or interruption/discontinuation of study treatment). When recording an AE resulting from a laboratory abnormality, the resulting medical condition rather than the abnormality itself should be recorded (e.g., record "thrombocytopenia" rather than "low platelets")
- Conditions known to have been present prior to the start of the study that increase in severity or frequency after administration of study drug should be recorded
- Signs, symptoms, or the clinical sequelae of a suspected drug interaction should be recorded

- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE)
- New and/or exacerbated symptoms of ALS should be recorded

Issues Not Considered AEs

- Medical or surgical procedures (e.g., endoscopy, appendectomy); rather, record the medical condition that led to a procedure (e.g., appendicitis)
- Situations where an untoward medical occurrence did not occur (e.g., social, diagnostic, or convenience admissions to a hospital)
- Fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not represent a clinically significant change after the first dose of study drug
- Abnormal laboratory or test findings that are not assessed by the Investigator as a clinically significant change after the first dose of study drug

7.1.2. Serious Adverse Events

An SAE is any AE that results in any of the following outcomes:

Death	AE resulted in death
Life threatening	The AE placed the patient at immediate risk of death. It does not refer to an event that hypothetically might have caused death if it were more severe, prolonged, or untreated.
Hospitalization	The AE required or prolonged an existing inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before the signing of informed consent in the study or routine check-ups are not SAEs by this criterion. Admission to palliative unit or hospice care facility is not considered to be a hospitalization. When in doubt as to whether 'hospitalization' occurred, consult the Medical Monitor.
Disability/ Incapacity	Resulted in a persistent or significant incapacity or substantial disruption of the patient's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) that may temporarily interfere with or prevent everyday life functions but do not constitute a substantial disruption.
Congenital Anomaly/Birth Defect	An adverse outcome in a child or fetus of a patient exposed to the molecule or study treatment regimen before conception or during pregnancy.

Important Medical Event	The AE did not meet any of the above criteria, but could have jeopardized the patient and might have required medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are invasive cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization. The development of drug dependency or drug abuse would also be examples of important medical events. If in doubt as to whether or not an event qualifies as a "medically significant event", consult the Sponsor's Medical Monitor.

7.2. Adverse Event Severity

The severity of AEs will be assessed using the National Cancer Institute (NCI) CTCAE, version 4.0 as defined below:

- Grade 1 (Mild) – Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2 (Moderate) – Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate activities of daily living
- Grade 3 (Severe) – Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- Grade 4 (Life-Threatening) – Life-threatening consequences; urgent intervention indicated
- Grade 5 (Fatal) – Death due to AE

Notes:

- AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious AE)
- Note that "life-threatening" in the criteria for "serious" has a more immediate definition than the CTCAE definition of "life-threatening". Thus, an AE may be CTCAE Grade 4 in severity and still not meet the SAE definition of "life-threatening".

7.3. Assessment of Causality to Study Treatment

The relationship of each AE to each study treatment [*tirasemtiv*] and [riluzole] should be evaluated by the Investigator using the following criteria:

Related	There is evidence to suggest a causal relationship between the drug and the AE, such as an event that is uncommon and known to be strongly associated with drug exposure (e.g. angioedema, hepatic injury, Stevens-Johnson Syndrome) an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g. tendon rupture)
Unrelated	Another cause of the AE is more plausible (e.g., due to underlying disease or occurs commonly in the study population), or a temporal sequence cannot be established with the onset of the AE and administration of the study treatment, or a causal relationship is considered biologically implausible

The assessment of causality will be based on the information available, and may be changed upon receipt of additional information.

7.4. Procedures for Eliciting and Recording Adverse Events

Investigator and study site personnel will report all AEs and SAEs whether elicited during patient questioning, discovered during physical examination, laboratory testing and/or other methods by recording them on the eCRF and/or SAE Form, as appropriate.

7.4.1. Eliciting Adverse Events

Use of an open-ended or non-directed method of questioning should be used at each study visit to elicit the reporting of AEs.

7.4.2. Recording of Adverse Events

When an AE occurs, the Investigator will review all documentation (e.g., hospital progress notes, laboratory and diagnostic reports) relevant to the event.

The following information should be recorded on the AE eCRF:

- Description including onset and resolution dates
- Whether serious criteria was met
- Severity
- Relationship to study treatment
- Outcome

7.4.3. Adverse Event Term

In general, the use of a unifying diagnosis is preferred to the listing out of individual signs/symptoms. Grouping of symptoms into a diagnosis should only be done if each component sign and/or symptom is a medically confirmed component of a diagnosis as evidenced by

standard medical textbooks. If any aspect of the sign or symptom does not fit into a classic pattern of the diagnosis, report the individual symptom as a separate AE.

7.4.4. Recording Serious Adverse Events

Record SAE terms on both the AE eCRF and an SAE Report Form. The following should be considered when recording SAEs:

- Death is an outcome. Record the event that resulted in death on both the SAE Form and AE eCRF
- For hospitalizations, surgical, or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself. Capture the procedure in the narrative description as part of the action taken in response to the illness

7.4.5. Pregnancy

Complete a Pregnancy Report Form and fax or email the completed form to the Sponsor's (or designee) Drug Safety Department within 24 hours of becoming aware of a pregnancy. Pregnancy, *per se*, will not be considered an AE in this study. However, a urine or serum pregnancy test should be performed if any female patient or partner of a male patient suspects that she has become pregnant during the time of signing of informed consent to within 10 weeks after the end of the study. If the test is positive, the pregnancy should be immediately reported to the Investigator and Sponsor. Any patient who becomes pregnant during the study is not eligible to continue in the study and should complete any end of study procedures at that time.

Complete pregnancy information, including the outcome of the pregnancy, should be collected in the source documents on the female patient or partner of a male patient (if she is willing). In the absence of complications, follow-up after delivery will be no longer than eight weeks. Abortion, whether accidental, therapeutic, or spontaneous, should be reported as an SAE. Congenital anomalies or birth defects should also be reported as defined by the 'serious' criterion (see definitions Section 7.1.2).

Any SAE occurring as a result of a post-study pregnancy and considered reasonably related to the investigational product by the Investigator should be reported to the Sponsor.

7.5. Reporting Period for Adverse Events

The safety reporting period for all AEs is from the first administration of study drug (Week -2 visit) through 28 days after the patient's last dose of study drug (Follow-Up Visit). However, all study protocol-related AEs are to be collected from the time of informed consent. All SAEs that occur after the safety reporting period and are considered study drug-related in the opinion of the Investigator should also be reported to the Sponsor.

All SAEs should be followed until significant changes return to baseline, until the condition stabilizes, or is no longer considered clinically significant by the Investigator, or until the patient dies, withdraws consent, or study closure. All non-serious AEs will be followed through the safety reporting period. Certain non-serious AEs of interest may be followed until resolution, return to baseline, or study closure.

7.5.1. Follow-Up of Adverse Events

After the initial recording of an AE, the Investigator should proactively follow the patient. Any non-serious AEs that are still on-going at the end of the study should be reviewed to determine if further follow-up is required. The Investigator will document on the AE eCRF any/all on-going non-serious AEs that will not be followed further (and the reason why) after the patient exits the study. If in doubt, the Investigator should consult the Sponsor's Medical Monitor.

The Sponsor may request that the Investigator perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of any AE.

7.6. Serious Adverse Events Require Immediate Reporting

Within 24 hours of the study site's knowledge of an SAE, Investigators are to report the event to the Sponsor, regardless of relationship of the event to the study drug.

For initial SAE reports, available details of the event are to be recorded on an SAE Report Form. At a minimum, the following should be included:

- Patient number
- Date of event onset
- Seriousness criterion or criteria
- Description of the event
- Study treatment
- Causality assessment

If not all information regarding an SAE is initially available, the site should not wait to receive additional information before reporting the event to the Sponsor within 24 hours.

8. STATISTICAL METHODS

8.1. General Considerations

8.1.1. General Approach

Summary statistics for continuous variables will include numbers of patients, means, medians, standard deviations, standard errors, minima, and maxima. For categorical variables, frequencies and percentages will be given. For time to event variables, the number of patients at risk, number of events, the median and 95% confidence intervals (CI) of the median, and quartiles will be provided by Kaplan Meier method. Assumptions for statistical models will be evaluated. If assumptions are substantially violated, alternative analysis methods will be considered. Missing data will not be imputed unless specified. Baseline is defined as the last available measurement taken before the first dose of study medication received in the open-label phase unless otherwise specified. Analyses will be conducted by study phase including open-label phase, first 24-week double blind placebo-controlled phase, 48-week double blind placebo-controlled phase, randomized withdrawal phase, and follow-up period.

8.1.2. Sample Size and Randomization

Approximately 600 patients will be enrolled in the study. Following the open-label phase, approximately 477 patients 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 treatment 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.

After patients complete the 48 weeks of the double-blind, placebo-controlled treatment, patients will be randomized to placebo or to continue their current active dose level of treatment in an allocation ratio of 1:1. Patients who are on placebo will be re-randomized only to placebo at the end of Week 48. Both randomizations will be stratified by riluzole use/non-use.

The common SD about the primary endpoint (the change from baseline to 24 weeks of the double-blind, placebo-controlled phase) 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 Table 13 . At this time, the Sponsor may consider increasing the sample size. For example, if the common SD is larger than 17 and equal to or less than 18, then consideration will 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).

Table 13: Sample Size Re-estimation 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 at Week 24	421	463	519

8.1.3. Data Monitoring Committee

An independent DMC (see Section 5.16) will periodically assess patient safety in an unblinded manner during the course of the study. The independent DMC will be provided unblinded data by an independent unblinded statistician from an independent biostatistical group. The DMC activities are described in the DMC charter.

8.2. Analysis Sets

8.2.1. Full Analysis Set (FAS)

The FAS will consist of all randomized patients who received at least one dose of study medication during randomized double-blind, placebo-controlled treatment, and had at least one post-randomization efficacy assessment. The FAS will be analyzed as randomized.

8.2.2. 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, have at least one post-baseline efficacy assessment during treatment with double-blind study drug, and have no major protocol violations.

8.2.3. Safety Analysis Set (SAS)

The SAS will consist of all patients who receive any study medication, including patients in the open-label phase.

8.2.4. Pharmacokinetic Evaluable Data Set (PKEDS)

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

8.3. Efficacy Endpoints

8.3.1. Primary Endpoint

The primary endpoint is the change from baseline to Week 24 of the double-blind, placebo-controlled phase in percent predicted SVC. The primary analysis will be conducted using all observed data during the randomized, double-blind, placebo-controlled phase without any missing data imputations and will be based on the FAS. To minimize the potential for missing data, patients who wish to discontinue study drug will be encouraged to continue study assessments and at minimum present for the 24 week assessments.

8.3.2. Secondary Endpoints

The following secondary endpoints will be analyzed in a closed testing procedure if the primary efficacy analysis is met as defined in Section 8.4.3.

- Change from baseline in the ALSFRS-R score of the three respiratory items of the ALSFRS-R (i.e., the sum of items 10, 11 and 12) at the end of 48 weeks of double-blind, placebo-controlled treatment
- Slope of mega-score of muscle strength during the 48 weeks of double-blind, placebo-controlled treatment
- 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 at the end of the 48 weeks of double-blind, placebo-controlled treatment
- 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
- Change from baseline in the ALSFRS-R total score to the end of 48 weeks of the double-blind, placebo-controlled treatment
- Time to the first use of mechanical ventilatory assistance or death during all 48 weeks of double-blind, placebo-controlled treatment

8.3.3. 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 the Statistical Analysis Plan. Additional tertiary endpoints of interest may be added to the Statistical Analysis Plan.

1. "Time to Event" analyses including:
    a. Time to the first occurrence of a decline in SVC to ≤ 50% predicted 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 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 either of 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 first three composite endpoints listed above will be analyzed with "first use of mechanical ventilatory assistance" in place of "respiratory insufficiency".

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
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. 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 mega-score of muscle strength)
   c. From the end of 24 weeks of the randomized, double-blind, placebo-controlled phase to the end of the double-blind, randomized, placebo-controlled phase at 48 weeks

5. Change 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. Change in percent predicted SVC from baseline to the end of Week 12 of the double-blind, placebo-controlled phase.

8.3.4. Additional Endpoints

1. ALSAQ-5 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 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

8.4. Statistical Analyses

8.4.1. Patient Disposition

The number of patients who are enrolled and receive open-label treatment but are not randomized will be presented. The number of patients who are randomized, who receive any double-blind study medication, who complete the study, and who prematurely discontinue from the study will be presented by treatment group and target dose level overall and for each phase separately. Reasons for premature discontinuation as recorded on the termination page of the eCRF will be summarized by treatment group and target dose level overall and for each phase separately.

8.4.2. Demographics and Other Baseline Characteristics

Patient demographics and other baseline characteristics will be summarized by treatment group and target dose level overall and by phase separately. To assess the comparability of treatment groups, demographic and baseline characteristics will be compared between the pooled active treatment dose levels and placebo for the FAS using Cochran-Mantel-Haenszel tests for binary categorical variables, van Elteren tests for ordinal categorical measures, or analysis of variance (ANOVA) for continuous variables, stratified by riluzole use/non-use and pooled site.

A demographic or baseline disease characteristic found to have a statistically significant (2-sided p-value ≤ 0.050) imbalance between the two treatment groups will be adjusted in supportive analyses of the primary efficacy endpoint.

8.4.3. Primary Efficacy 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 in the FAS randomized to placebo and those randomized to *tirasemtiv* (pooled three target dose levels) during the placebo-controlled double-blind treatment. The analysis will be performed using a repeated-measures mixed model which will include terms of treatment, baseline, pooled center, visit, and riluzole use/non-use as well as interaction terms of treatment-by-visit and baseline-by-

visit with an unstructured covariance matrix, using all observed data during the 24-week, double-blind, placebo-controlled phase without any missing data imputations. The treatment variable will be introduced to the mixed model with 4 categories including 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.

The sites will be pooled by country and then by criteria to be determined if there are more than 40 patients enrolled in one country. The detailed algorithm will be determined and submitted to the regulatory authorities after the enrollment completes and before the database lock for Week 24 placebo-controlled phase.

8.4.4. Supportive Efficacy Analyses

As a key supportive analysis, the primary efficacy endpoint will be analyzed for the FAS using rank transformation repeated measures analysis of covariance (ANCOVA) including the same terms in the primary efficacy analysis. Ranks will be applied to all observed changes from baseline (regardless of visits) for continuous variables in the model in case large variance heterogeneity or inflation of variance from extreme outliers is observed. Based on the extent of outliers in the blinded data review when all patients complete the treatment at Week 24 before database lock, the rank-based analysis (or a more rigorous randomized-based version for within visit rankings) will be conducted.

As supportive analyses, the primary efficacy endpoint will be analyzed for the FAS and the PPS using the same method as the primary efficacy analysis, including terms of unbalanced baseline characteristics and the treatment-by-factor interactions.

8.4.5. Secondary and Tertiary Efficacy Analyses

All analyses will be performed with the methods below for all secondary or tertiary endpoints for the first 24-week double-blind placebo-controlled phase and the 48-week double-blind placebo-controlled phase separately. For endpoints related to skeletal muscle function, the interaction of treatment-by-riluzole will also be included in the models.

Secondary or tertiary endpoints of change from baseline parameters will be analyzed using the same method as the primary efficacy analysis.

For binary variables, the proportion of patients with an event will be provided. Odds ratios and their 95% CIs as well as p-values will be calculated using conditional logistic regression for treatment difference between *tirasemtiv* and placebo, stratified for pooled site with terms of baseline, treatment, and riluzole use/non-use in the model.

For time to event variables, a proportional hazards Cox regression model will be used to estimate the hazards ratio and its 95% CI between the two treatment groups, including treatment and covariates of baseline SVC value, pooled site, and riluzole use/non-use.

Slope endpoints will be analyzed using a mixed model which will include treatment, the baseline value, pooled site, time, and riluzole use/non-use as well as interaction terms of treatment-by-baseline, treatment-by-time, assuming random slope effect. Slope endpoints will be compared between the patients randomized to all *tirasemtiv* target dose levels during the double-blind, placebo-controlled phase and the patients who were randomized to placebo during the double-

blind, placebo-controlled phase for the first 24-week double-blind placebo-controlled phase and the 48-week double-blind placebo-controlled phase separately.

8.4.6. Other Supportive Efficacy 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 double-blind placebo-controlled phase)/placebo (4 weeks of randomized withdrawal phase) versus placebo (48 weeks of double-blind placebo-controlled phase)/placebo (4 weeks of randomized withdrawal phase) for the randomized withdrawal phase. The 95% CIs will be calculated for the difference in LSM between the placebo/placebo group and the *tirasemtiv*/placebo group.

8.4.7. 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 in the FAS.

8.4.8. Hypothesis Testing and 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. The methodology of the closed testing procedure is fully described in the statistical analysis plan.

Comparisons of dose groups for the primary and secondary endpoints will be considered exploratory and used to supportively confirm which driving dose(s) that has the effect on these endpoints versus placebo.

8.4.9. Sensitivity Analysis

Several sensitivity analyses will be conducted. 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. 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. When imputed data no longer have compatibility with parametric assumptions, ANCOVA based on ranks subsequent to imputations will be used. Second, the primary efficacy analysis will be repeated in all randomized patients in the first 24 weeks of placebo-controlled phase.

8.4.10. Subgroup Efficacy Analyses

Analyses of the primary efficacy endpoint and the secondary endpoints evaluating the change from baseline in the ALSFRS-R score of the three respiratory items of the ALSFRS-R at the end of 48 weeks of double-blind, placebo-controlled treatment and the slope of mega-score of muscle strength during the 48 weeks of double-blind, placebo-controlled treatment phase will be conducted in subgroups based on the medians of percent predicted SVC, body weight, and BMI at baseline, riluzole use/non-use, baseline ALSFRS-R, time since first symptom, time since diagnosis of ALS, by randomized target dose level, by actual maintenance dose established during the double-blind placebo-controlled phase, sex, age group (<65, ≥ 65 years old), race, geographic region (i.e., Europe versus North America), anatomic site of disease onset (bulbar versus limb), and other subgroups as may be specified in the SAP, in the FAS. The reduced sample size within each subgroup will reduce the power of the statistical tests, so significant treatment effects within each subgroup are not expected.

8.5. Safety Analysis

Safety analyses will be based on the SAS overall and by the phases of the study (i.e., open-label phase; the first 24 weeks of the double-blind, placebo-controlled phase; all 48 weeks of the double-blind, placebo-controlled phase; the double-blind, placebo-controlled *tirasemtiv* withdrawal phase; and until the end of the follow-up period as well as subgroup analyses by riluzole use/non-use.

8.5.1. Adverse Events

A TEAE is an AE with an onset after initiation of study drug dosing, or an AE present at initiation of study drug dosing that worsens in severity after initiation of study drug dosing. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Preferred Terms and grouped by System Organ Class. The version of the MedDRA dictionary will be noted in the report. AEs will be classified according to CTCAE grade.

TEAEs will be assigned to the open-label phase treatment, to the randomized target dose level, or to the dose level administered immediately prior to the onset of the TEAE during the double-blind treatment. TEAEs that begin during the open-label phase but continue without an increase in severity after randomization into the double-blind, placebo-controlled, treatment phase will be assigned to the randomized, double-blind treatment only if they persist without an increase in severity beyond 96 hours after the first dose of double-blind, placebo-controlled study medication. In BENEFIT-ALS, this convention minimized the assignment to double-blind placebo of those TEAEs that began during treatment with open-label *tirasemtiv* but resolved shortly after the initiation of treatment with double-blind placebo while appropriately assigning to double-blind *tirasemtiv* those TEAEs that began during treatment with open-label *tirasemtiv* that continued past the initiation of treatment with double-blind *tirasemtiv*.

If a TEAE continues after a change in dose level without an increase in intensity, it will be assigned only to the dose level at which it initiated. If a TEAE continues and worsens in intensity after a change in dose level, it will be assigned both to the dose level at which it initiated and to the dose level at which it worsened. If a TEAE resolves during continued treatment at the same dose level at which it initiated but then recurs following a change in dose

level, it will be assigned both to the dose level at which it initiated and resolved and again at the dose level at which it re-initiated.

The number and percentage of patients with TEAEs will be summarized by system organ class, preferred term, dose level, and treatment overall and by phase. The number and percentage of patients reporting TEAEs will be tabulated by system organ class, preferred term, dose level, treatment, and severity overall and by phase. On patient-based AE tables, for a specific TEAE, the patient will be counted only once under the most severe grade if the TEAE is reported more than once per patient per dose level and treatment. The above AE summaries will also be performed by riluzole use and non-use. Additionally, the number and percentage of patients with TEAEs will be summarized for the open-label phase, by system organ class, preferred term, comparing patients who later received active *tirasemtiv* to those who received placebo during the randomized double-blind treatment. Further tabulation by severity for the open-label phase will also be provided. Similar TEAE summaries will be provided for the double-blind, placebo-controlled phase and the *tirasemtiv* withdrawal phase as well. The above TEAE summaries will be repeated by randomized treatment and dose level.

Only TEAEs occurring from the first dose through 28 days after the last dose of study drug will be summarized. All AEs will be included in patient listings.

8.5.2. Other Safety Assessments

The death incidence density will be provided by treatment group, adjusting for patients' actual treatment duration. The ratio of death incidence density and 95% CI in *tirasemtiv* relative to placebo will be calculated using Poisson regression. Weight change from baseline will be evaluated by using the same method as the primary analysis. Clinical laboratory data, vital signs, ECGs, neurological exams, physical exams, and falls assessments will be descriptively summarized by dose level and treatment overall and by phase.

8.5.3. Suicidality Assessments

Suicidality ideation and its intensity will be summarized descriptively by treatment and dose level overall and by phase.

8.6. Study Drug Exposure

Study drug exposure will be summarized. The distribution of exposure by randomized dose level will be tabulated overall and by phase.

8.7. Concomitant Medication

Concomitant medications taken between seven days prior to the first dose of study drug throughout 28 days after the last dose of study drug will be summarized and classified by drug class and preferred name by treatment, dose levels overall and for each study phase, using the World Health Organization (WHO) Drug dictionary in the most current version when the study enrollment starts. The version of the WHO Drug dictionary will be noted in the clinical study report.

8.8. Pharmacokinetic Analysis

PK analysis will be based on the PKEDS. Descriptive statistics (arithmetic mean, SD, median, minimal, maximum, geometric mean, and coefficient of variation) will be presented for the concentration of *tirasemtiv*, its metabolites, and for riluzole by treatment group and study visit.

8.9. Pharmacodynamic Analysis

Pharmacodynamic (PD) analysis may be performed to explore the relationship between *tirasemtiv* exposure and efficacy and safety endpoints.

8.10. Change in Statistical Methods

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

9. ADMINISTRATIVE ASPECTS

9.1. Change in Protocol

There will be no alterations in the protocol without agreement between the Sponsor and the Principal Investigator (PI).

There will be no alterations in the protocol without the express written approval of the Sponsor, Investigator, and the IRB/EC/REB (see Form FDA 1572).

9.2. Initiation Visit

Prior to the start of the clinical study, the representative(s) of the Sponsor will meet with the Investigator(s) and appropriate clinical staff to familiarize the Investigator and clinical staff with the materials necessary for conducting the clinical study.

9.3. Disclosure

All information provided regarding the study, as well as all information collected/documented during the course of the study, will be regarded as confidential. The Investigator agrees not to disclose such information in any way without prior written permission from the Sponsor.

Any publication of the results, either in part or in total (e.g., articles in journals or newspapers, oral presentations, abstracts, etc.) by the Investigator(s) or their representative(s), shall require prior notification and review, within a reasonable time frame, by the Sponsor, and cannot be made in violation of the Sponsor's confidentiality restrictions or to the detriment of the Sponsor's intellectual property rights.

9.4. Monitoring

The Sponsor will designate site monitors who will be responsible for monitoring this clinical trial. The site monitor will monitor the study conduct, proper eCRF and source documentation completion and retention, and accurate study drug accountability. To this end, the monitor will visit the study site at suitable intervals and be in frequent contact through verbal and written communication. It is essential that the site monitor have access to all documents (related to the study and the individual participants) at any time these are requested. In turn, the site monitor will adhere to all requirements for patient confidentiality as outlined in the ICF. The Investigator and other study personnel will be expected to cooperate with the site monitor, to be available during a portion of the monitoring visit to answer questions, and to provide any missing information.

9.5. Institutional Review Board / Ethics Committee / Research Ethics Board

In accordance with the US Code of Federal Regulations, 21 CFR 56, the protocol, advertisement (if applicable), and ICF will be submitted to the IRB/EC/REB for review and subsequent written approval by the IRB/EC/REB must be received before proceeding. The Sponsor will supply relevant material for the Investigator to submit to the IRB/EC/REB for the protocol's review and

approval. Verification of the IRB/EC/REB unconditional approval of the protocol and the written ICF statement will be transmitted to the Investigator.

The IRB/EC/REB will be informed by the Investigator of subsequent protocol amendments and of serious and unexpected AEs. Approval for protocol amendments will be transmitted in writing to the Investigator. If requested, the Investigator will permit audits by the IRB/EC/REB and regulatory inspections by providing direct access to source data/documents.

The Investigator will provide the IRB/EC/REB with progress reports at appropriate intervals (not to exceed one year) and a Study Progress Report following the completion, termination, or discontinuation of the Investigator's participation in the study.

9.6. Informed Consent

Written informed consent for the study will be obtained from all patients before protocol-specific procedures are carried out. The ICF generated by the Investigator (or designee) will be approved (along with the protocol) by the IRB/EC/REB and will be acceptable to the Sponsor.

The Investigator (or designee) will explain the nature of the study and the action of the test product. The patients will be informed that participation is voluntary and that they can withdraw from the study at any time. In accordance with 21 CFR 50, informed consent shall be documented by the use of a written ICF approved by the IRB/EC/REB and will be signed by the patient prior to protocol-specific procedures being performed. The patient will be given a copy of the signed consent, and the original will be maintained with the patient's records. A copy of the IRB/EC/REB-approved site-specific ICF must be sent to the Sponsor (or designee).

9.7. Records

The results from data collected during the study will be recorded in the patient's eCRF. To maintain confidentiality, the patients will be identified only by numbers.

The completed eCRFs will be transferred to the Sponsor or designee. All source documents, records, and reports will be retained by the study site in accordance with 21 CFR 312.62(c). All primary data, or copies thereof (e.g., laboratory records, source documents, correspondence, photographs, and computer records), which are a result of the original observations and activities of the study and are necessary for the reconstruction and evaluation of any study report, will be retained in the study site archives.

9.8. Reference to Declaration of Helsinki/Basic Principles

The study procedures outlined in this protocol will be conducted in accordance with the CFR governing Protection of Human Subjects (21 CFR 50), Financial Disclosure by Clinical Investigators (21 CFR 54), IRBs (21 CFR 56), Investigational New Drug Application (21 CFR 312), and Applications for FDA Approval to Market a New Drug (21 CFR 314), as appropriate. As such, these sections of U.S. Title 21 CFR, along with the applicable ICH Guidelines, are commonly known as Good Clinical Practices (GCP), which are consistent with the Declaration of Helsinki, 1996.

10. REFERENCES

Hwee, D., A. Kennedy, et al. (2014) "Fast Skeletal Muscle Troponin Activator tirasemtiv Increases Muscle Function and Performance in the B6SJL-SOD1G93A ALS Mouse Model." PLoS One DOI: 10.1371/journal.pone.0096921.

Kiernan, M., S. Vucic, et al. (2011). "Amyotrophic lateral sclerosis." Lancet **377**(9769): 942-955.

Lacomblez, L., G. Bensimon, et al. (1996). "Dose-ranging study of riluzole in amyotrophic lateral sclerosis." The Lancet **347**: 1425-1431.

Russell, A., J. Hartman, et al. (2012). "Activation of fast skeletal muscle troponin as a potential therapeutic approach for treating neuromuscular diseases." Nat Med **18**(3): 452-455.

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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. SUBSTRATES OF CYP2B6, 2C8, 2C9, AND 2C19

2B6	2C8	2C9	2C19
artemisinin	amodiaquine	**NSAIDs:**	**PPIs:**
bupropion	cerivastatin	diclofenac	esomeprazole
cyclophosphamide	paclitaxel	ibuprofen	lansoprazole
efavirenz	repaglinide	lornoxicam	omeprazole
ifosphamide	sorafenib	meloxicam	pantoprazole
ketamine	torsemide	S-naproxen→Nor	**Anti-epileptics:**
meperidine		piroxicam	diazepam→Nor
methadone		suprofen	phenytoin(O)
nevirapine		**Oral Hypoglycemic**	S-mephenytoin
propafol		tolbutamide	phenobarbitone
selegiline		glipizide	**Other:**
sorafenib		**Angiotensin II Blockers:**	amitriptyline
		losartan	carisoprodol
		irbesartan	citalopram
		Sulfonylureas:	chloramphenicol
		glyburide	clomipramine
		glibenclamide	clopidogrel
		glipizide	cyclophosphamide
		glimepiride	hexobarbital
		tolbutamide	imipramine N-DeME
		Other:	indomethacin
		amitriptyline	labetalol
		celecoxib	R-mephobarbital
		fluoxetine	moclobemide
		fluvastatin	nelfinavir
		glyburide	nilutamide
		nateglinide	primidone
		phenytoin-4-OH	progesterone
		rosiglitazone	proguanil
		tamoxifen	propranolol
		torsemide	teniposide
		valproic acid	R-warfarin→8-OH
		S-warfarin	voriconazole
		zakirlukast	

http://medicine.iupui.edu/clinpharm/ddis/main-table/

APPENDIX C. SUBSTRATES, INHIBITORS AND INDUCERS OF CYP1A2

Substrates	Inhibitors	Inducers
amitriptyline	fluvoxamine***	broccoli
caffeine	ciprofloxacin***	brussel sprouts
clomipramine	cimetidine*	carbanazepine
clozapine	amiodarone	char-grilled meat
cyclobenzaprine	efavirenz	insulin
duloxetine	fluoroquinolones	methylcholanthrene
estradiol	fluvoxamine	modafinil
fluvoxamine	furafylline	nafcillin
haloperidol	interferon	beta-naphthoflavone
imipramine N-DeMe	methoxsalen	omeprazole
mexiletine	mibefradil	rifampin
nabumetone	ticlopidine	tobacco
naproxen		
olanzapine		
ondansetron		
phenacetin1		
acetaminophen→NAPQI		
propranolol		
riluzole		
ropivacaine		
tacrine		
theophylline		
tizanidine		
triamterene		
verapamil		
(R)warfarin		
zileuton		
zolmitriptan		

***strong inhibitor, *weak inhibitor
http://medicine.iupui.edu/clinpharm/ddis/main-table/