A Phase 2a, Randomized, Double-blind, Placebo-controlled, Two Period, Crossover Study to Assess the Effect of CK-2127107 on Physical Function in Subjects with Chronic Obstructive Pulmonary Disease

ISN/Protocol 3318-CL-3002

ClinicalTrials.gov Identifier: NCT02662582

Date of Protocol v3.1: 29 Nov 2017

Sponsor: Astellas Pharma Global Development, Inc. (APGD)

1 Astellas Way

Northbrook, IL 60062

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Version 3.1

Incorporating Non-Substantial Amendment 1 [See Attachment 1]

29 November 2017

IND 127897

Sponsor:

Astellas Pharma Global Development, Inc. (APGD)
1 Astellas Way
Northbrook, IL 60062

Protocol History:
Version 1.0 [24Nov2015]
Version 2.0 Incorporating Substantial Amendment 1 [22Mar2016]
Version 3.0 Incorporating Substantial Amendment 2 [13Mar2017]

Investigator: Investigator information is on file at Astellas

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I. SIGNATURES

1. SPONSOR'S SIGNATURE

Required signatures (e.g., Protocol authors, Sponsor's reviewers and contributors, etc.) are located in [Section 14, Sponsor's Signatures]; e-signatures (when applicable) are located at the end of this document.

2 COORDINATING INVESTIGATOR'S SIGNATURE

A Phase 2a, Randomized, Double-blind, Placebo-controlled, Two Period, Crossover Study to Assess the Effect of CK-2127107 on Physical Function in Subjects with Chronic Obstructive Pulmonary Disease

ISN/Protocol 3318-CL-3002

Version 3.1 / Incorporating Non-Substantial Amendment 1

29 November 2017

I have read all pages of this clinical study protocol for which Astellas is the Sponsor. I agree that it contains all the information required to conduct this study.

Coordinating Investigator:

Signature: _____ Date (DD Mmm YYYY)

PPD

Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center

Printed Name: _____

Address: _____

_____

3 INVESTIGATOR'S SIGNATURE

A Phase 2a, Randomized, Double-blind, Placebo-controlled, Two Period, Crossover Study to Assess the Effect of CK-2127107 on Physical Function in Subjects with Chronic Obstructive Pulmonary Disease

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29 November 2017

I have read all pages of this clinical study protocol for which Astellas is the Sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with ICH GCP guidelines and applicable local regulations. I will also ensure that subinvestigator(s) and other relevant members of my staff have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: .....

Date (DD Mmm YYYY)

Printed Name: .....

Address: .....

.....

II. CONTACT DETAILS OF KEY SPONSOR'S PERSONNEL

24h-Contact for Serious Adverse Events (SAEs) [See Section 5.5.5]	PPD **, Immunology, Infectious Disease, CNS and Pain** **Astellas Pharma Global Development, Inc.** **Please fax or email the SAE Worksheet to:** **North America Fax: 1-888-396-3750** **North America Alternate Fax: 1-847-317-1241** **International Fax Number: +44-800-471-5263** **Email: safety-us@astellas.com**
Medical Monitor/Medical Expert:	PPD Immunology, Infectious Disease, CNS and Pain Astellas Pharma Global Development, Inc. PPD
Clinical Research Contact:	PPD , Clinical Science Astellas Pharma Global Development, Inc. PPD

III. LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS

List of Abbreviations

Abbreviations	Description of Abbreviations
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
AST	aspartate aminotransferase
ATS	American Thoracic Society
BMI	body mass index
C-PPAC	clinical visit version of PROactive physical activity in COPD
Ca2+	calcium
COPD	chronic obstructive pulmonary disease
CRO	contract research organization
CSR	clinical study report
CWR	constant work rate
CYP	cytochrome P450
ECG	electrocardiogram
eCRF	electronic case report form
ED	early discontinuation
EDC	electronic data capture
EMG	electromyogram
EOT	end of treatment
ERS	European Respiratory Society
FAS	full analysis set
FEV_1	forced expiratory volume in 1 second
FUV	follow-up visit
FVC	forced vital capacity
GCP	Good Clinical Practice
GGT	γ-glutamyl transferase
GMP	Good Manufacturing Practice
IB	investigator's brochure
IC	inspiratory capacity
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IET	incremental exercise test
INR	international normalized ratio
IRB	institutional review board
IRT	Interactive Response Technology
LA-CRF	liver abnormality case report form
LFT	liver function tests
LHRH	luteinizing hormone-releasing hormone

Abbreviations	Description of Abbreviations
MATE	multidrug and toxin extrusion
MEP	maximal expiratory pressure
MIP	maximal inspiratory pressure
mMRC	Modified Medical Research Council
NOAEL	no observed adverse effect level
OAT	organic anion transporter
OCT	organic cation transporter
PKAS	pharmacokinetic analysis set
PRO	patient reported outcome
QoL	quality of life
RPE	rating perceived exertion
RV	residual volume
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SDD	spray dried dispersion
SF-36	Short Form-36
SGRQ-C	St. George's Respiratory Questionnaire for COPD patients
SOP	standard operating procedure
SPPB	short physical performance battery
SUSAR	suspected unexpected serious adverse reaction
TBL	total bilirubin
TEAE	treatment-emergent adverse event
THC	tetrahydrocannabinol
TLC	total lung capacity
ULN	upper limit of normal
V_E	ventilation
V_E/VCO_2	ventilatory equivalent for carbon dioxide
VO_2	oxygen uptake
WHO	World Health Organization
WR	work rate

Definition of Key Study Terms

Terms	Definition of Terms
Baseline	Observed values/findings which are regarded observed starting point for comparison.
Enroll	To register or enter into a clinical trial. NOTE: Once a subject has been enrolled, the clinical trial protocol applies to the subject.
Intervention	The drug, therapy or process under investigation in a clinical study that is believed to have an effect on outcomes of interest in a study (e.g., health-related quality of life, efficacy, safety, pharmacoeconomics).
Investigational period	Period of time where major interests of protocol objectives are observed, and where the study drug or comparative drug (sometimes without randomization) is usually given to a subject, and continues until the last assessment after completing administration of the study drug or comparative drug.
Randomization	The process of assigning trial subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screen failure	Potential subject who did not meet 1 or more criteria required for participation in a trial.
Screening	A process of active consideration of potential subjects for enrollment in a trial.
Screening period	Period of time before entering the investigational period, usually from the time of starting a subject signing consent until just before the study drug or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first site initiation date to the last site completing the study.
Variable	Any quantity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

IV. SYNOPSIS

Date and Version # of Protocol Synopsis:	29 November 2017 / Version 3.1
Sponsor: Astellas Pharma Global Development Inc. (APGD)	**Protocol Number:** 3318-CL-3002
Name of Study Drug: CK-2127107	**Phase of Development:** 2a

Title of Study:
A Phase 2a, Randomized, Double-blind, Placebo-controlled, Two Period, Crossover Study to Assess the Effect of CK-2127107 on Physical Function in Subjects with Chronic Obstructive Pulmonary Disease

Planned Study Period:
From 1Q2016 to 1Q2017

Study Objective(s):

Primary Objective

- To assess the effect of CK-2127107 relative to placebo on cycle ergometer exercise tolerance, assessed as change from period baseline in constant work rate (CWR) endurance time, utilizing a breath-by-breath metabolic measurement system with integrated electrocardiogram (ECG). The time to intolerance is assessed by a stopwatch and verified from electronic recordings of the cycle ergometer.

Secondary Objectives

- To assess cardiopulmonary and neuromuscular effects of CK-2127107 relative to placebo on:
  - The change from period baseline in oxygen uptake (VO_2), ventilation (V_E), ventilatory equivalent for carbon dioxide (V_E/VCO_2) and other breath-by-breath cardiometabolic variables, inspiratory capacity (IC) and perceived exertion for dyspnea and leg discomfort (Borg CR10), at isotime* and peak during CWR test
  - Activation of accessory respiratory muscles (by electromyogram [EMG]) at isotime* and peak exercise during CWR test
- To assess the effect of CK-2127107 on resting spirometry relative to placebo
- To assess the safety and tolerability of CK-2127107
- To assess the pharmacokinetics of CK-2127107

*Isotime is the shortest duration of all of the CWR tests performed before and after treatment, not including screening.

Exploratory Objectives

- To explore the effects of CK-2127107 relative to placebo on:
  - Patient reported outcomes (PROs)
  - Physical performance via a short physical performance battery (SPPB)
  - Respiratory muscle strength by maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP)

Planned Total Number of Study Centers and Location(s):
Approximately 4 sites located in the United States and possibly Canada or the United Kingdom

Study Population:

Male and female subjects aged 40 to 75 years old with COPD

Number of Subjects to be Enrolled/Randomized:

Approximately 40 subjects are planned to be randomized.

Study Design Overview:

This is a phase 2a, randomized, double-blind, placebo-controlled, two period, crossover study to assess the potential effects on physical function and safety of CK-2127107 in subjects with COPD.

Enrolled subjects will be randomly assigned to 1 of 2 treatment sequences and will receive both CK-2127107 and matching placebo over 2 treatment periods as per the following schedule.

Sequence	Treatment Period 1	Treatment Period 2
1	CK-2127107	Placebo
2	Placebo	CK-2127107

Each treatment period consists of 14 days dosing of CK-2127107 or matching placebo. Each medication period is separated by a 14-day washout period. The total study duration including screening period and follow-up visit (FUV) for an individual subject will be approximately 12 weeks (see Flow Chart and Schedule of Assessments).

Subjects will attend up to 3 screening visits (between days -28 and day -1) prior to randomization at day 1 of treatment period 1. At screening visits 1 and 2 between days -28 and -8, each subject will undergo standard screening procedures: Subjects will undergo spirometry before and after administration of a bronchodilator. Postbronchodilator forced expiratory volume in 1 second (FEV_1) expressed as a percent of predicted and as the ratio of FEV_1/forced vital capacity (FVC) will be an inclusion criterion. Subjects will perform 1 incremental exercise test (IET) on a bicycle to characterize the individual subject's cardiopulmonary responses and also to determine the peak work rate (WR). After the IET, a CWR exercise test will be performed, which is set at a level that brings the subject to intolerance at between 4 to 8 minutes using a WR calculated from IET results. The CWR test will be completed at least 24 hours after IET. At visit 3 a weekly PRO assessment (clinical visit version of PROactive physical activity in COPD [C-PPAC]) will be completed using a 7-day recall online 12-item questionnaire. Visit 3 will be considered optional if the subject successfully completes the CWR at visit 2. On day 1 (visit 4) at baseline, all subjects will complete postbronchodilator spirometry, respiratory muscle strength tests (MIP and MEP), a resting ECG, CWR test with cardiopulmonary and respiratory muscle EMG measurements, quality of life (QoL) assessments (St. George's Respiratory Questionnaire for COPD patients [SGRQ-C], Short Form-36 [SF-36]), and SPPB to establish period baseline measurements, and will be randomly assigned to 1 of 2 treatment sequences in a 1:1 ratio.

To achieve steady-state exposure to CK-2127107, subjects will receive CK-2127107 (500 mg) or matching placebo twice daily for 14 days, except on day 1 and day 14. On day 1, subjects will take active or placebo drug once in the evening, and on day 14, subjects will take active or placebo drug once in the morning. The subjects will return to the study site again on day 14 (visit 6) for the main assessment day (MAD) visit for treatment period 1. Physical activity assessment will be completed using a 7-day (days 7 to 13) recall online 12-item questionnaire.

The exercise testing and the evaluation of the respiratory system will be conducted in the same chronologic order (spirometry, MIP/MEP, CWR, SPPB) at day 1 and day 14. Subsequent visits for baseline (visit 7) and treatment period 2 will then follow as per the schedule of assessments.

During the study, blood samples will also be collected at predefined time points for the assessment of plasma concentrations of CK-2127107 and possible metabolite(s) (if applicable). One optional blood sample for pharmacogenomics will also be taken prior to first dose at day 1 of treatment period 1 (predose) if the subject specifically consents to this sample collection.

For all subjects, safety will be assessed through the monitoring of adverse events (AEs), concomitant medication, vital signs (including blood pressure, heart rate and body temperature), clinical laboratory assessments, physical examination and ECG parameters. Stopping rules for renal and liver safety are implemented.

Upon completion of 2 treatment periods each subject will attend a FUV (visit 10), 7 to 14 days after the last dose or early withdrawal.

An overview and planning of all assessments can be found in the flow chart and schedule of assessments.

Inclusion/Exclusion Criteria:

Inclusion:

A subject is eligible for the study if all of the following apply:

1. Institutional review board (IRB)-/independent ethics committee (IEC)-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Male or female aged 40 to 75 inclusive, at the signing of the informed consent.
3. Subject has a body mass index (BMI) of 18 to 35 kg/m^2 inclusive
4. Subject must have all of the following:
    a. Clinical diagnosis of moderate to severe COPD, with a postbronchodilator FEV_1/FVC ratio $< 70\%$ and $30\% \leq FEV_1 < 80\%$ predicted at screening. The predicted values for normal spirometry will be those recommended by the American Thoracic Society (ATS) / European Respiratory Society (ERS) [Miller et al, 2005].
    b. General stable health with no change in medication (including non-COPD agents and dietary aids/food supplements) within 2 weeks prior to screening, no systemic corticosteroid administration (topical or inhaled corticosteroids are allowed) within 6 weeks prior to screening, no exacerbations or hospitalization within 6 weeks prior to screening.
    c. Current or ex-smokers with a smoking history of at least 10 pack years*.
    d. Grade of 2 or 3 on the Modified Medical Research Council (mMRC) Dyspnea Scale at screening:
        - Grade 2: walks slower than people of the same age on the level because of breathlessness or has to stop for breath when walking at own pace on the level
        - Grade 3: stops for breath after walking about 100 meters or after a few minutes on the level
5. Subject is able to complete technically acceptable respiratory muscle strength tests, spirometry, physical performance test and exercise tests.
6. This criterion has been deleted.

7. Female subject must either:
    - Be of non-child bearing potential:
        - Postmenopausal (defined as at least 1 year without any menses) prior to screening, or
        - Documented surgically sterile
    - Or, if of childbearing potential,
        - Agree not to try to become pregnant during the study and for 28 days after the last dose,
        - And have a negative serum pregnancy test at screening,
        - And, if heterosexually active, agree to consistently use 2 forms of highly-effective birth control** (at least 1 of which must be a barrier method) starting at screening, throughout the study, and for 28 days after the last dose.
8. Female subject must agree not to breastfeed starting at screening and throughout the study and for 28 days after the last dose.
9. Female subject must not donate ova starting at screening, throughout the study and for 28 days after the last dose.
10. Male subject and their female spouse/partners who are of childbearing potential must be using highly effective form of contraception consisting of 2 forms of birth control** (at least 1 of which must be a barrier method) starting at screening, and continuing throughout the study and for 90 days after the last dose.
11. Male subject must not donate sperm starting at screening, throughout the study and for 90 days after the last dose.
12. Subject agrees not to participate in another interventional study from screening through the FUV of the study.

*Pack year is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. For example, 1 pack year is equal to smoking 1 pack per day for 1 year, or 2 packs per day for half a year, and so on.

**Highly effective forms of birth control include:
- Consistent and correct usage of established oral contraception
- Established intrauterine device (IUD) or intrauterine system (IUS)
- Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository

Waivers to the Inclusion Criteria will NOT be allowed.

Exclusion:

Subjects will be excluded from participation if any of the following apply:

1. Subject has previously enrolled in a clinical study of CK-2127107.
2. Subject has any clinically significant abnormality following the investigator's review of the physical examination, ECG and protocol-defined clinical laboratory tests at screening. A significant abnormality is defined as an abnormality which, in the opinion of the investigator, may (i) put the subject at risk because of participation in the study, (ii) influence the results of the study or (iii) cause concern regarding the subject's ability to participate in the study.
3. Subject has any of the liver function tests (LFTs; i.e., aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], γ-glutamyl transferase [GGT] and/or total bilirubin [TBL]) above 1.5 times the upper limit of normal (ULN) at screening. These assessments may be repeated once at the investigator's discretion (within the screening window).
4. Subject has an estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m^2 by the Cockcroft-Gault equation at screening.

5. Subject has a serious cardiovascular disease, including a current New York Heart Association (NYHA) class III or IV congestive heart failure or clinically significant valvular disease, history of cardiac arrest, uncontrolled angina or arrhythmia, untreated serious conduction disorder (e.g., third-degree heart block), or acute myocardial ischemic condition suspected on the ECG at screening (e.g., ST-segment elevation, ST-segment depressions > 2 mm).
6. Subject has had a myocardial infarction or other acute coronary syndrome, major heart surgery (i.e., valve replacement or bypass surgery), stroke, deep vein thrombosis or pulmonary embolus in the 6 months prior to screening.
7. Subject has known active tuberculosis.
8. Subject has undergone thoracotomy with pulmonary resection (except for sub-lobar resection).
9. Subject has resting pulse < 40 bpm or > 100 bpm; resting systolic blood pressure > 160 mm Hg or < 90 mm Hg; resting diastolic blood pressure > 100 mm Hg at screening. These assessments may be repeated once at the investigator's discretion (within the screening window).
10. Subject desaturates to SpO_2 < 85% for at least 1 minute on screening IET.
11. Subject has a limitation of exercise performance as a result of factors other than fatigue or exertional dyspnea/shortness of breath (considered to be due to COPD), such as arthritis in the leg, angina pectoris, heart failure, claudication or morbid obesity.
12. Subject has a CWR cycle ergometry endurance time less than 4 or greater than 8 minutes after WR adjustment procedures at screening visit 2.
13. Subject has used the following drugs within 14 days prior to day -1:
    a. Strong cytochrome P450 (CYP)3A4 inhibitor (e.g., itraconazole, clarithromycin).
    b. Strong CYP3A4 inducer (e.g., barbiturates, rifampin).
14. Subject has hemoglobin (Hb) concentration below 10.0 g/dL at screening.
15. Subject has a cancer requiring treatment currently or in the past 3 years (except primary non-melanoma skin cancer, carcinoma in situ or cancers that have an excellent prognosis such as early stage breast or prostate cancer).
16. Subject giving a history of asthma, allergic rhinitis or atopy shall be evaluated by the investigator to determine whether the subject's predominant diagnosis is COPD rather than asthma.
17. Subject has neurological conditions or neuromuscular diseases that are causing impaired muscle function or mobility.
18. Subject has a current diagnosis of schizophrenia, other psychotic disorders or bipolar disorder.
19. Subject in the active phase of pulmonary rehabilitation or had completed pulmonary rehabilitation or exercise training within the 13 weeks prior to screening.
20. Subject has severe and/or uncontrolled medical conditions that could interfere with the study (e.g., severe neurological deficit after stroke, developed diabetic peripheral neuropathy, respiratory diseases requiring daytime supplemental oxygen, infection, gastrointestinal disorder, uncontrolled pain or any other non-stable illness) as judged by the medical investigator.
21. Subject has a known history of positive test for hepatitis B surface antigen (HBsAg) or hepatitis C antibody or history of a positive test for human immunodeficiency virus (HIV) infection.
22. Subject has a history of alcoholism or drug/chemical substance abuse within 2 years prior to screening.

23. Subject has used any medications known to affect physical function or muscle mass including androgen supplements, anti-androgens (such as luteinizing hormone-releasing hormone [LHRH] agonists), anti-estrogen (tamoxifen, etc.), recombinant human growth hormone (rhGH), oral beta adrenergic agonists, megestrol acetate, dronabinol or other drugs which, in the opinion of the investigator, might influence physical function or muscle mass within 6 weeks prior to screening.
24. Subject has participated in any interventional clinical study or has been treated with any investigational drugs within 28 days or 5 half-lives whichever is longer, prior to the initiation of screening.
25. Subject has any other condition that in the opinion of the investigator precludes the subject's participation in the trial.

Waivers to the Exclusion Criteria will NOT be allowed.

Investigational Product(s):
CK-2127107-C 250 mg tablets**,** film-coated

Dose(s):
1000 mg (500 mg twice daily)

Mode of Administration:
Oral doses to be taken in the morning and evening within 2 hours after meals (approximately 12 hours apart).

Comparative Drug(s):
Matching placebo tablets

Dose(s):
Matching placebo for CK-2127107 (500 mg twice daily)

Mode of Administration:
Oral doses to be taken in the morning and evening within 2 hours after meals (approximately 12 hours apart).

Rescue Therapy:
In the event of bronchospasm, albuterol is allowed to be self-administered by the subject, except for testing days. Subjects requiring rescue albuterol on testing days (beyond the dose administered with spirometry) before completing all assessments must return to the clinic as soon as possible to repeat the entire study day.

NOTE: During the scheduled spirometry assessments the subjects will receive 2 puffs of albuterol in the laboratory 10 to 15 minutes prior to the spirometry assessment on each study day. Requirement of additional "rescue" doses would indicate that that the subject's disease is not stable.

Dose(s):
180 μg (2 puffs)

Mode of Administration:
Inhaled by mouth every 4 to 6 hours; not to exceed 12 inhalations/24 hours

Concomitant Medication/Food Restrictions or Requirements:

The following concomitant medications and treatments will not be allowed during the study:

- Systemic corticosteroids
- Strong CYP3A4 inhibitors (e.g., itraconazole, clarithromycin, grapefruit juice, Seville oranges)
- CYP3A4 inducers (e.g., barbiturates, rifampin, St. John's wort),
- Cannabis/tetrahydrocannabinol (THC)-based medication,
- Skeletal muscle relaxants,
- OCT1/OCT2 substrates: oxaliplatin, pindolol, varencicline, pilisicainide and dofetilide,
- Any medications known to affect physical function or muscle mass including androgen supplements, anti-androgens (such as LHRH agonists), anti-estrogen (tamoxifen, etc.), rhGH, oral beta adrenergic agonists, megestrol acetate, dronabinol or other drugs which, in the opinion of the investigator, might influence physical function or muscle mass.

Caution is recommended for concomitant administration of the organic cation transporter OCT1 substrates (e.g., aciclovir, ganciclovir) and in particular the OCT1/2 substrate metformin (antidiabetic), as CK-2127107 may have the potential to inhibit OCT1 and OCT2-mediated transport.

Chronic medications including COPD and non-COPD agents and dietary aids/food supplements are permitted, but change in the dose regimen or dosage, or starting any new medication is not allowed. All concomitant medication usage will be noted and recorded during each study visit.

Albuterol (180 μg) is to be administered by inhalation 10 to 15 minutes prior to spirometry assessment on each study day.

In case non-permitted treatment is necessary, withdrawal of the subject from the study should be considered by the authorized medical personnel.

Duration of Treatment:

Two 14-day treatment periods separated by a 14-day wash out period.

Endpoints for Evaluation:

Primary:

- Change from period baseline in CWR endurance time relative to placebo

Secondary:

- Change from period baseline in VO_2, V_E, V_E/VCO_2, IC, as well as other cardiometabolic variables and perceived exertion for dyspnea and leg discomfort (Borg CR10) at isotime* and peak during CWR as compared to placebo
- Change from period baseline in activation of accessory respiratory muscles (by EMG) at isotime* and peak exercise during CWR compared to placebo
- Change in resting spirometry compared to placebo

*Isotime is the shortest duration of all of the CWR tests performed before and after treatment, not including screening.

Exploratory:

- Change from period baseline in QoL outcomes (SGRQ-C, SF-36) relative to placebo
- Change from baseline in physical activity using a 7-day recall online 12-item questionnaire
- Change from period baseline in physical performance tests battery score (SPPB) relative to placebo
- Change from period baseline in MIP and MEP relative to placebo. This test is performed before exercise testing and is part of the resting assessment.

Safety:

- Adverse events (AEs) /serious adverse events (SAEs)
- Vital signs (blood pressure, heart rate, body temperature)
- Clinical laboratory tests (serum chemistry, hematology and urinalysis)
- 12-lead ECG

Pharmacokinetics:

- Pharmacokinetics of CK-2127107 and possible metabolite(s) (if applicable):

  Day 1 (only treatment period 2): predose plasma concentration
  Day 14 (of both treatment period 1 and 2): C_{trough} and C_{6h}

Statistical Methods:

Sample Size Justification:

A sample size of 40 subjects will provide 84% power to detect a difference of 60 seconds in CWR duration from period baseline for CK-2127107 versus placebo with a 1-sided alpha error of 0.05, assuming the standard deviation for change from period baseline is 100 seconds. In order to maintain the statistical power of 80%, approximately 36 subjects would need to complete period 1. Should the discontinuation rate in the first treatment period exceed 10% (approximately 5 subjects), additional subjects will need to be recruited to account for the discontinued subjects.

Efficacy:

Change from period baseline in CWR endurance time at the end of each period will be analyzed by a mixed effect model for the primary endpoint. The model includes subject as a random effect and treatment and period as fixed effects. Analysis of secondary endpoints will be performed based on a similar model as the primary endpoint.

Pharmacokinetics:

Descriptive statistics (e.g., number of subjects, mean, standard deviation, minimum, median, maximum, coefficient of variation and geometric mean) will be provided for pharmacokinetic parameters of CK-2127107 and possible metabolite(s) (if applicable).

Pharmacodynamics:

Not applicable.

Safety:

All variables will be summarized descriptively by treatment.

Interim Analyses:

Not applicable.

V. FLOW CHART AND SCHEDULE OF ASSESSMENTS

Figure 1

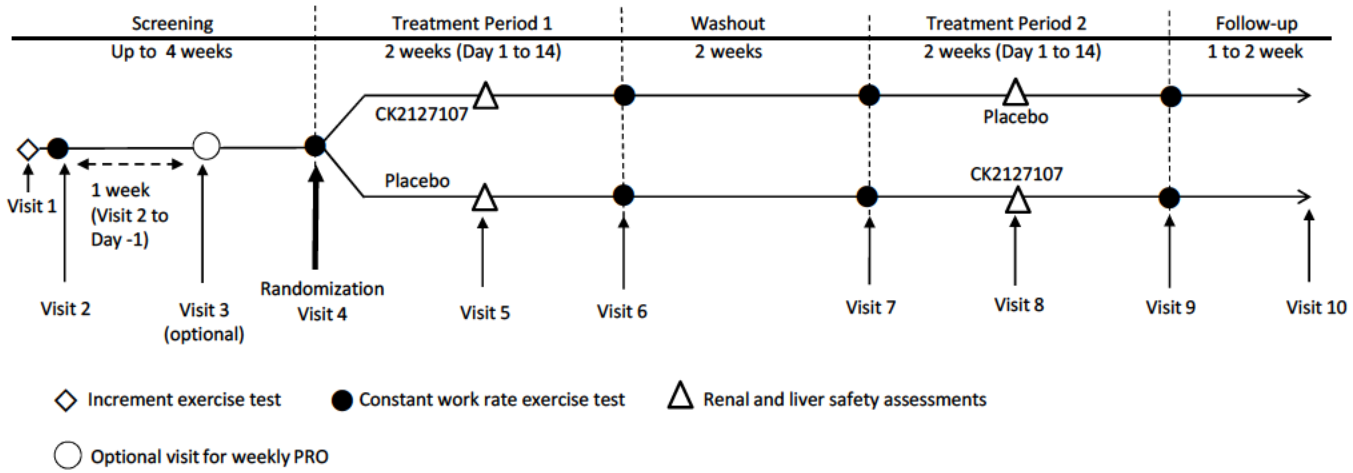

Table 1 Schedule of Assessments

Assessments	**Screening**			**Treatment Period 1**			**Washout**	**Treatment Period 2**			**Follow-up**
Day	-28 to -9	-27 to -8	-19 to -1	1	6	14	14 days	1	6	EOT[a]/ ED	7 days after EOT/ED
Window	-	-	-	-	+3	+3	+7	-	+3	+3	+7
Visit Number	1	2	3[b]	4	5	6	-	7	8	9	10
Informed consent	X										
Inclusion/Exclusion	X										
Medical history	X			X				X			
Demographics	X										
Drug and alcohol screen[c]	X			X				X			
Randomization				X							
Dispense/Collect study drug				X		X		X		X	
Administration of study drug[d]				←	———	→		←	———	→	
Physical examination[e]	X			X		X		X		X	X
Vital signs[f]	X			X		X		X		X	X
Hematology	X			X		X		X		X	X
Serum chemistries	X			X	X[g]	X		X	X[f]	X	X
Serology	X										
Urinalysis	X			X		X		X		X	X
Serum pregnancy test	X									X	X
Spirometry[h]	X			X		X		X		X	X
Respiratory muscle strength tests (MIP, MEP)				X		X		X		X	
12-lead ECG	X			X		X		X		X	X
CWR exercise test		X[i]		X		X		X		X	
QoL outcomes (SGRQ-C, SF-36)				X		X		X		X	
Physical performance test (SPPB)				X		X		X		X	
IET	X										

Table continued on the next page

Assessments	**Screening**			**Treatment Period 1**			**Washout**	**Treatment Period 2**			**Follow-up**
Day	-28 to -9	-27 to -8	-19 to -1	1	6	14	14 days	1	6	EOT[a]/ ED	7 days after EOT/ED
Window	-	-	-	-	+3	+3	+7	-	+3	+3	+7
Visit Number	1	2	3[b]	4	5	6	-	7	8	9	10
PRO (C-PPAC)				X		X					
Blood sampling for pharmacokinetics[j]						Predose 6h		Predose		Predose 6h	
Optional blood sampling for pharmacogenomics[k]				X							
Concomitant medication	X	X	X	X	X	X[l]	X	X	X[l]	X	X
Adverse events assessment	X	X	X	X	X	X[l]	X	X	X[l]	X	X

C-PPAC: clinical visit version of PROactive physical activity in COPD; CWR: constant work rate; ECG: electrocardiogram; ED: early discontinuation; EOT: end of treatment; IET: incremental exercise test; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; PRO: patient reported outcome; SF-36: Short Form-36; SGRQ-C: St. George's Respiratory Questionnaire for COPD; SPPB: short physical performance battery

a. EOT: day 14 of treatment period 2.

b. Visit 3 will be considered optional if the subject successfully completes the CWR at visit 2. Subjects who do not attend visit 3 will resume their visit schedule with visit 4.

c. A standard urine screen for drugs of abuse (e.g., cannabinoids, cocaine and amphetamines) and benzodiazepines, and blood, urine, or breathalyzer alcohol screen will be performed by a central laboratory.

d. The time of daily study drug dosing is to be recorded in the paper diary. Subjects will take study drugs as follows: once a day after dinner at day 1, twice a day within 2 hours after breakfast and dinner between day 2 and day 13 and once a day within 2 hours after breakfast at day 14. In order to collect the trough pharmacokinetic sample on day 14, the subject is to wait and take the morning dose at the site after pharmacokinetic sample collection has been performed.

e. A full physical examination will be performed at screening visit 1 and follow-up visit 10. Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal, and neuromuscular. At visits 4, 6, 7, and 9, a symptom-directed physical exam will be performed.

f. Vital signs include blood pressure, heart rate and body temperature.

g. Specifically for the monitoring of creatinine, blood urea nitrogen (BUN), cystatin C and liver enzymes (renal and liver safety assessments) at visits 5 and 8.

h. At screening visit, subjects perform spirometry both pre and postbronchodilator, but only postbronchodilator thereafter: Subjects will receive 2 puffs of albuterol in the laboratory 10 to 15 minutes prior to the spirometry assessment on each study day.

Footnotes are continued on the next page

i. At screening CWR will be completed at least 24 hours after IET at visit 2 and may be retested within the next 5 days at the investigator's discretion (within the visit window) if target exercise duration is not obtained. If this cannot be achieved with 2 adjustments (a total of 3 CWR tests), the subject will be classified as a screen failure and no additional assessments completed. All exercise tests (IET and CWR) will record heart rate and ECG simultaneously with breath-by-breath gas exchange and ventilatory variables during all exercise performance. Borg CR10 is performed with CWR at all of the time points shown in the schedule. The Electromyogram (EMG) is also performed with the CWR, but only at visits 4, 6, 7 and 9/EOT.

j. Blood samples for pharmacokinetics of CK-2127107 and possible metabolite(s) (if applicable) will be collected from EVERY subject. A single pharmacokinetic sample will be collected at each of the following time points: prior to evening dose at day 1 of treatment period 2, prior to morning dose (trough) and then approximately 6 hours postmorning dose at day 14 of both treatment period 1 and 2.

k. Sample to be collected 1 time prior to first dose at day 1 of treatment period 1.

l. If a subject experiences an AE or change in concomitant medications during days 7 to 13 or washout, they should call the study site to inform them of this change.

1 INTRODUCTION

CK-2127107 is a small molecule activator of the fast-skeletal-muscle troponin complex, a sarcomere-directed therapy intended to improve skeletal muscle function in conditions associated with muscular weakness. Although it is derived from a different structural class of compounds, CK-2127107 has the same mechanism of action as that of tirasemtiv (formerly known as CK-2017357), another fast-skeletal-muscle activator currently in a phase 3 study of subjects with amyotrophic lateral sclerosis and for which evidence of a pharmacodynamic effect already exists [Shefner et al, 2012].

CK-2127107 selectively activates the fast-skeletal-muscle troponin complex by increasing its sensitivity to calcium, thereby increasing skeletal muscle force in response to neuronal input and delaying the onset and reducing the degree of muscle fatigue. CK-2127107 is selective for the troponin complex in fast skeletal muscle and does not activate the slow skeletal troponin complex or the cardiac troponin complex. CK-2127107 is orally bioavailable in nonclinical species and healthy humans; consequently, oral administration is the intended route of administration in subjects.

CK-2127107 is hypothesized to reduce the energy cost of exercise by reducing the adenosine triphosphate demands associated with calcium ion pumping.

For more details about the investigational product, refer to the current Investigator's Brochure (IB).

1.1 Background

Chronic obstructive pulmonary disease (COPD) is an obstructive and progressive airway disease. The leading cause of COPD is smoking. According to the World Health Organization (WHO), COPD is the fifth leading cause of death in the world, with approximately 3 million deaths per annum, or 5% of deaths [WHO, 2015]. People with COPD experience limitations in expiratory airflow to their lungs, resulting in a significant reduction in physical activity and psychological problems, all of which contribute to the patient's disability and poor quality of life. Standard treatments are bronchodilators, corticosteroids and pulmonary rehabilitation.

Overall, poor exercise capacity in COPD patients or overall low levels of physical activity are indicated to correlate with increased mortality [Waschki et al, 2011; Garcia-Aymerich et al, 2006; Oga et al, 2003]. The patho-physiological processes underlying the relationship between physical activity and survival in chronic diseases such as COPD are complex and not well understood [Aguilaniu, 2010]. To date, only a few drugs have been shown to enhance exercise capacity of patients with COPD, including beta2 agonists, tiotropium and low-density gas mixtures [O'Donnell et al, 2004; Palange et al, 2004; Celli et al, 2003]. There is currently no pharmacological treatment available for skeletal muscle dysfunction and wasting in chronic pulmonary disorders. Troponin modulators and calcium sensitizers such as tirasemtiv and CK-2127107 sensitize fast skeletal muscle to calcium (Ca2+) and slow the rate of Ca2+ release from the regulatory troponin complex of fast skeletal muscle, resulting in a sensitization of the contractile apparatus to calcium.

The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have recently updated their statement on limb muscle dysfunction in COPD, recognizing this to be a key systemic consequence of COPD [Maltais et al, 2014]. According to this official statement of the ATS, assessment of limb muscle function could serve to identify patients at increased risk of poor clinical outcomes such as exercise intolerance and premature mortality. Current treatment options for limb muscle dysfunction include rehabilitative exercise training or neuromuscular electrical stimulations, particularly in patients who are severely impaired or suffering exacerbations.

A variety of interventions have been tested in order to improve muscle function in patients with COPD such as oxygen, nutritional supplements with or without exercise, anabolic steroids such as testosterone, growth hormone and other bioactive nutrients. Exercise training appears to improve muscle strength, endurance and fatigability, but the magnitude of response to exercise is highly variable.

In contrast to the respiratory system, limb muscles demonstrate plasticity in response to exercise and could therefore provide an opportunity for novel drug targets. The aim is to find treatment options to improve limb muscle function and reduce overall disability [Ribeiro et al, 2013]. CK-2127107 is hypothesized to selectively activate the fast skeletal muscle troponin complex by increasing its sensitivity to calcium. CK-2127107 slows the rate of calcium ion release from the regulatory troponin complex of fast skeletal muscle resulting in a sensitization of the contractile apparatus to calcium. It is selective for fast skeletal muscle over slow or cardiac muscle and may produce benefit in patients by increasing skeletal muscle force in response to neuronal input and delaying the onset and potentially reducing the degree of muscle fatigue.

There are a variety of exercise tests available in order to quantify changes in exercise capacity in COPD patients such as timed walk tests (6-minute walk test, shuttle walk test) or incremental cardiopulmonary exercise testing and constant work rate exercise testing. Constant work rate cycle ergometry has been found useful to detect improvements in exercise tolerance after an intervention [Borel B et al, 2013; Casaburi R, 2005; Oga T et al, 2000]. In the currently proposed study CK-2127107 is hypothesized to improve the physiological ability to sustain endurance exercise by demonstrating an increase in constant work rate (CWR) endurance time.

1.2 Nonclinical and Clinical Data

CK-2127107 is currently in 2 phase 2 clinical evaluations for chronic obstructive pulmonary disease and spinal muscular atrophy following completion of 5 phase 1 studies. Nonclinical evaluations include a series of safety studies in rat and monkey, including single- and repeat-dose (28-day and 13-week) toxicity studies, a core battery of safety pharmacology studies, reproductive and developmental toxicity studies, in vitro and in vivo genotoxicity tests, and an in vivo phototoxicity study. To date, suspension formulations of CK-2127107, either of the crystalline active pharmaceutical ingredient (API) of an amorphous spray dried dispersion (SDD) and a tablet formulation comprised of API, have been used in nonclinical and clinical studies.

1.2.1 Nonclinical Data

Dosing of CK-2127107 increased running performance in healthy rats and rats with heart failure produced by myocardial infarction under slowly accelerating conditions on a rotarod apparatus. Additionally, studies were conducted in human diaphragm obtained from biopsies. CK-2127107 restored the reduced contractile force of muscle fibers from subjects on mechanical ventilation to that of control muscle fibers. The pharmacological profile of CK-2127107, acting as a direct functional activator of fast skeletal muscle, could be of benefit to subjects with a wide variety of disorders characterized by muscle weakness.

The toxicology studies conducted mainly in rats and cynomolgus monkeys, selected on the basis of in vitro metabolism studies with CK-2127107 demonstrate a favorable safety profile.

In 28-day repeat-dose toxicity and toxicokinetic studies, the no observed adverse effect level (NOAEL) for CK-2127107 SDD was 600 mg/kg per day, the highest dose level evaluated, in both rats and monkeys.

In both species, there were modest reversible changes in clinical chemistries, hematology, and coagulation that, in the absence of correlating macroscopic or microscopic findings, were considered to represent non-adverse effects of the test article. While no microscopic findings were noted in monkeys, some reversible non-adverse microscopic changes were noted in the kidneys, liver and thyroid of rats. These findings are recognized rat-specific patterns of histological changes.

A 13-week repeat-dose toxicity and toxicokinetic study was conducted in rats at doses of 0, 10, 30, 150 and 600 mg/kg per day by oral gavage. All animals survived until scheduled termination. There were microscopic findings observed in the liver, kidney, pancreas, adrenal gland and thyroid gland. Organ weight and macroscopic/microscopic findings were reversible or partially reversible following the 4-week recovery period. None of the findings observed were considered adverse and the NOAEL was considered to be at the highest repeat dose tested of 600 mg/kg per day for both sexes.

A 13-week repeat-dose toxicity and toxicokinetic study was conducted in cynomolgus monkeys at doses of 0, 30, 150 and 600 mg/kg per day by oral gavage. CK-2127107-related clinical chemistry changes included increases in creatinine and triglyceride concentrations in both sexes at dose levels ≥ 150 mg/kg and changes in urine color with an increased incidence in urine bilirubin in males and females across all dose levels. At 600 mg/kg per day, there were increases in the incidence of urine protein and, in a few females, detectable urine glucose. CK-2127107-related microscopic findings of minimal to mild follicular cell hypertrophy and hyperplasia were observed in the thyroid gland of males at dose levels ≥ 150 mg/kg per day. Significant increases in liver weights were observed in females at 600 mg/kg per day and in males at dose levels ≥ 150 mg/kg per day. These liver weights had no microscopic correlate. All of these findings were reversible after the 4-week recovery period. None of the findings in this study were considered adverse and the NOAEL for CK-2127107 was considered to be 600 mg/kg per day for both sexes.

The core battery of safety pharmacology studies conducted with CK-2127107 indicated no functional changes in vital organs or systems which are likely to be of importance in clinical testing of CK-2127107. Similarly, an effect of CK-2127107 on the human Ether-à-go-go Related Gene (hERG) channel was noted (half-maximal inhibitory concentration [IC_{50}] = 230.8 μM), but no effect was noted on the QT interval in cynomolgus monkeys.

No effects of CK-2127107 on fertility or early embryonic development were observed in rats treated at 0, 30, 150 and 600 mg/kg per day. In an embryo-fetal development toxicity study in rats (0, 30, 150 and 600 mg/kg/day), a delayed ossification ascribable to a maternal toxicity (decreases in body weight and food consumption) was observed at 150 and 600 mg/kg, but no teratogenicity was indicated. In an embryo-fetal development toxicity study in rabbits (0, 10, 30 and 100 mg/kg/day), resorption and a decreased fetal body weight ascribable to a maternal toxicity (decreases in body weight and food consumption) were noted at 100 mg/kg, but no teratogenic potential was indicated.

The results of testing CK-2127107 and CK-2127106 in the bacterial reverse mutation, in vitro cytogenetic and in vivo rodent bone marrow micronucleus (in this last test, CK-2127106 is an abundant metabolite formed after dosing CK-2127107) studies indicated a lack of genotoxic hazard.

CK-2127107 has detectable absorbance in the UVA-UVB/visible range, but an in vitro phototoxicity study was negative.

While CK-2127107 is a substrate for cytochrome P450 (CYP)3A4, CK-2127107 did not inhibit CYP isozyme activity (1A2, 2C9, 2C19, 2D6 or 3A4) in vitro, either directly or in a time-dependent manner. CK-2127107 (10 μM) did not activate transcription of human pregnane X receptor or aryl hydrocarbon receptor-regulated genes (i.e., CYP3A4 and CYP1A genes) compared with control compounds in vitro indicating no evidence for induction of these enzymes. CK-2127107 inhibited organic cation transporter (OCT)1, OCT2, multidrug and toxin extrusion (MATE)1, and organic anion transporter (OAT)1 activities in a concentration-dependent manner. CK-2127107 inhibited OCT1 and OCT2 with (IC_{50}) values of 2.63 μmol/L and 2.59 μmol/L, respectively. The IC_{50} values for other transporters such as MATE1, OAT1, MATE2-K, OAT2, and OAT3 activity were > 100 μmol/L. The IC_{50} values for the metabolite, CK-2127106, against the same group of transporters were > 100 μmol/L.

1.2.2 Clinical Data

Five phase 1 studies have been completed. Two studies were primarily directed toward evaluating the pharmacokinetics and tolerability of single- and multiple ascending doses of CK-2127107. In a first-in-human study conducted in 35 healthy men (Study CY 5011), single oral doses of CK-2127107 SDD suspension ranging from 30 to 4000 mg were all well tolerated and no maximum tolerated dose was established. In Study CY 5012, multidose administration of 300 and 500 mg twice daily for either 10 or 17 days was well tolerated by 59 young and elderly healthy subjects and allowed the steady-state pharmacokinetic profile to be described. A review of electrocardiograms (ECGs) obtained at baseline and various

time points at all dose levels revealed no clinically significant and/or obvious dose-dependent changes in QT interval vs. placebo.

Two other studies were directed toward comparing the pharmacokinetics and tolerability of different physical forms or formulations of CK-2127107. In Study CY 5014, the relative oral bioavailability and pharmacokinetic profiles of the stabilized amorphous form of CK-2127107 as an SDD in suspension and the crystalline form of CK-2127107 API in suspension were compared following single doses of 300 mg and 1000 mg administered to 25 healthy men. Both dose levels were well tolerated. Similar oral bioavailability of CK-2127107 SDD and API was demonstrated at the 300 mg dose level, but not at the 1000 mg dose level. Across the dose range 300 to 1000 mg, SDD but not API was dose proportional for both AUC_{inf} and C_{max}. In Study CY 5015, the pharmacokinetics of a tablet formulated with CK-2127107 API was compared to CK-2127107 API suspension and found to have adequate bioavailability for use in future studies.

Finally, a 4-way crossover single dose pharmacokinetic/pharmacodynamic study (Study CY 5013) performed with placebo, 300, 1000 and 3000 mg demonstrated a dose- and concentration-dependent increase in the force-frequency relationship of the tibialis anterior muscle with transcutaneous electric stimulation of the common peroneal nerve.

The primary objective of this study was to determine the change in the force-frequency profile and its relationship to dose and plasma concentrations when CK-2127107 is administered orally to healthy male subjects.

The effect of CK-2127107 on changes from baseline in force-frequency response showed significant increases relative to placebo. At 1, 3, 5 and 7 hours after administration of the 1000 and 3000 mg doses of CK-2127107, the changes from baseline in normalized AUC force (based on the sum of all frequencies) were statistically significantly greater than changes following placebo dosing. The 300 mg dose of CK-2127107 produced changes from baseline that were significantly different from changes following placebo dosing at 1 and 7 hours postdose. For all dose groups, the greatest changes from baseline were observed at 3 hours postdose, which coincided with C_{max}.

The terminal half-life ($t_{1/2}$) of CK-2127107 was generally around 12 hours with a t_{max} of 2 to 3 hours. The increase in exposure was largely dose-proportional although exposure was more variable at doses ≥ 3000 mg. The exposure of CK-2127107 was higher at steady-state than after a single dose on day 1 with an accumulation ratio of approximately 3 to 4 at 300 mg and 4 to 5 at 500 mg (CY 5012). The inactive metabolite (CK-2127106) was present at 4% to 19% of the parent in plasma. Exposure to CK-2127106 was higher at steady-state than after a single dose with mean accumulation ratios > 20, consistent with its longer $t_{1/2}$ of about 40 hours (CY 5012). C_{max} and AUC values increased somewhat greater than dose-proportionally following multiple dosing. There were no differences in pharmacokinetic parameters between young and elderly subjects but mean exposure was slightly higher in women versus men.

CK-2127107 was well tolerated at all dose levels with no serious adverse events (SAEs) or discontinuations due to an adverse event (AE). The most common (> 10%) AEs observed with CK-2127107 versus placebo were dizziness and headache. Gastrointestinal symptoms as a whole also exceeded the 10% incidence but the individual symptoms did not (abdominal pain, diarrhea, nausea, vomiting). Dizziness and headache appeared to be dose-related and their incidence increased at doses of 2250 mg and above. None of the AEs were classified as severe. Four subjects in the multiple dose study, CY 5012 (1 treated with placebo and 3 treated with CK-2127107 500 mg), had an AE of "increased alanine aminotransferase (ALT)/hepatic enzymes"; an additional 3 subjects (all treated with CK-2127107 500 mg) had at least 1 elevated liver enzyme during treatment with study drug that was not reported as an AE. Of these 7 subjects, some of whom also had modest bilirubin elevations, none had values that met Hy's Law criteria. Other laboratory tests remained normal and consistent with baseline values following dosing, with the exception of elevated serum creatinine values in most subjects treated with CK-2127107. This was observed consistently across all studies. CK-2127107 has been demonstrated to inhibit OCT2, a mediator of renal tubular secretion of creatinine in the human kidney, with an IC_{50} of 2.59 µmol/L [see the last paragraph of Section 1.2.1, above]. Consequently, it is believed that inhibition of renal tubular OCT2 by CK-2127107 is the most likely reason for these generally small and reversible increases in serum creatinine during treatment with CK-2127107.

1.3 Summary of Key Safety Information for Study Drugs

There were no differences in steady-state pharmacokinetics of CK-2127107 between young and elderly subjects. Thus no dose adjustment in the elderly appears necessary. Exposure to the inactive metabolite, CK-2127106, was higher in the elderly, but the clinical significance of this finding, if any, is not known.

Although not observed in single dose studies, a total of 7 subjects in the multidose study CY 5012 had an abnormal liver function test (LFT), whether reported as a treatment-emergent adverse event (TEAE) or as a laboratory value. Of the 7 subjects, some also had modest bilirubin elevations, but none had values that met Hy's Law criteria for drug-induced liver injury (ALT or aspartate aminotransferase [AST] > 3 x the upper limit of normal [ULN], associated with total bilirubin [TBL] > 2 x ULN, with or without increases in alkaline phosphatase [ALP]). The clinical significance of these findings is not known at this time. In nonclinical toxicology studies conducted out to 13 weeks in rat and monkey, there have been no adverse histological changes noted in the liver up to the highest dose of 600 mg/kg, which is also the NOAEL. See [Appendix 12.3 Liver Safety Monitoring and Assessment] for detailed information on liver abnormalities, monitoring and assessment.

A concentration-dependent increase in serum creatinine levels during treatment with CK-2127107 that returned to baseline after discontinuation of treatment has also been observed. No renal toxicity had been observed in nonclinical toxicology studies although a similar increase in creatinine during dosing was noted in monkey and rats after 13 weeks of exposure that was reversible. At this time, it is believed that this finding is likely due to inhibition of renal tubular OCT2 by CK-2127107, as OCT2 mediates renal tubular creatinine

secretion (see Section 1.2.1 above) a mechanism which has been reported with other drugs [Agarwal, 1993; Hilbrands et al, 1993; Payne, 1993; van Acker et al, 1993; Schutzer et al, 2010]. Interference with the creatinine assay by CK-2127107 or 1 of its metabolites could also be a potential factor underlying these transient increases in serum creatinine [Ducharme et al, 1993].

1.4 Risk-Benefit Assessment

Given the early stage of development and focus on obtaining initial safety data for CK-2127107, the AE profile seen to date appears acceptable for proceeding into clinical studies in subjects with muscle-wasting diseases as well as additional studies in healthy subjects as needed. Both LFTs and serum creatinine will be obtained at baseline and during dosing with CK-2127107 in order to further characterize these findings. In addition, stopping rules have been implemented to ensure the safety of subjects enrolled into this study [Section 5.4.2.2].

Study 3318-CL-3002 will include COPD patients in stable condition meeting detailed entry criteria and who are required to follow restrictions for concomitant medications.

As a safety precaution, patients shall be discontinued from the study drug* should their serum creatinine demonstrate an increase of 1.5 to 2.0 times above baseline (Common Terminology Criteria for Adverse Events [CTCAE] grade 1) in concordance with an increase in serum cystatin C levels.

* Patients should be evaluated first for potential other reasons of elevated serum creatinine and cystatin C levels (i.e., dehydration, concomitant medication or any underlying pathology that could explain the increase in renal markers).

2 STUDY OBJECTIVE(S), DESIGN AND ENDPOINTS

2.1 Study Objectives

To assess the effect of CK-2127107 relative to placebo on cycle ergometer exercise tolerance, assessed as change from period baseline in CWR endurance time, utilizing a breath-by-breath metabolic measurement system with integrated electrocardiogram (ECG). The time to intolerance is assessed by a stopwatch and verified from electronic recordings of the cycle ergometer.

There are several secondary objectives and exploratory objectives for this study.

Secondary Objectives

- To assess cardiopulmonary and neuromuscular effects of CK-2127107 relative to placebo on:
  - The change from period baseline in oxygen uptake (VO_2), ventilation (V_E), ventilatory equivalent for carbon dioxide (V_E/VCO_2) and other breath-by-breath cardiometabolic variables, inspiratory capacity (IC) and perceived exertion for dyspnea and leg discomfort (Borg CR10), at isotime* and peak during CWR test

  - Activation of accessory respiratory muscles (by electromyogram [EMG]) at isotime* and peak exercise during CWR test
- To assess the effect of CK-2127107 on resting spirometry relative to placebo
- To assess the safety and tolerability of CK-2127107
- To assess the pharmacokinetics of CK-2127107

*Isotime is the shortest duration of all of the CWR tests performed before and after treatment, not including screening.

Exploratory Objectives

- To explore the effects of CK-2127107 relative to placebo on:
  - Patient reported outcomes (PROs)
  - Physical performance via a short physical performance battery (SPPB)
  - Respiratory muscle strength by maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP)

2.2 Study Design and Dose Rationale

2.2.1 Study Design

This is a phase 2a, randomized, double-blind, placebo-controlled, two period, crossover study to assess the potential effects on physical function and safety of CK-2127107 in subjects with COPD. Approximately 40 subjects are planned to be randomly assigned to 1 of 2 treatment sequences and will receive both CK-2127107 and matching placebo over 2 treatment periods as per the following schedule [Table 2]. If the discontinuation rate in the first treatment period exceeds 10%, additional subjects will be recruited to ensure a total of at least 36 subjects completing the study.

Table 2 Treatment Sequence

Sequence	Treatment Period 1	Treatment Period 2
1	CK-2127107	Placebo
2	Placebo	CK-2127107

Each treatment period consists of 14 days dosing of CK-2127107 or matching placebo. Each medication period is separated by a 14-day washout period. The total study duration including screening period and follow-up visit (FUV) for an individual subject will be approximately 12 weeks (see Flow Chart [Figure 1] and Schedule of Assessments [Table 1]).

Subjects will attend up to 3 screening visits (between day -28 and day -1) prior to randomization at day 1 of treatment period 1. At screening (visits 1 and 2 between days -28 to -8) each subject will undergo standard procedures including resting spirometry before and after administration of a bronchodilator. Postbronchodilator forced expiratory volume in 1 second (FEV_1) expressed as a percent of predicted, and as the ratio of FEV_1/forced vital capacity (FVC) will be an inclusion criterion. Subjects will perform resting ECG and 1 incremental exercise test (IET) to characterize the individual subject's aerobic capacity (VO_2 peak), lactate threshold, ventilatory efficiency (V_E/VCO_2 nadir) and peak work rate (WR), followed by a CWR exercise test, both assessed on a cycle ergometer.

The CWR test will be completed at visit 2 at least 24 hours after the IET. The CWR exercise test will be performed at 80% peak WR (rounded to the nearest 5 watts). This screening test is used to confirm the correct WR selection for the rest of the study and to familiarize the subject with the testing procedure. Subjects are expected to tolerate this WR for durations in the range of 4 to 8 minutes. Establishing a WR that will achieve this specific duration is critical if constant work rate testing is deemed to detect treatment effects in interventional studies [Casaburi R, 2005; Puente-Maestu et al, 2016]. In the event that the tolerable duration is outside this range, a second CWR is performed within the next 5 days at ± 5 watts to determine whether this adjusted WR will result in an endurance time in the desired range. If this cannot be achieved with 2 adjustments (a total of 3 CWR tests), the subject will be classified as a screen failure and no additional assessments completed. Once the WR is established that leads to intolerance in 4 to 8 minutes, all subsequent tests use the WR that led to intolerance. No additional WR adjustments can be made once the WR intolerance level is set.

On day 1 (visit 4) at baseline, all subjects will complete postbronchodilator spirometry, respiratory muscle strength tests (MIP and MEP), a resting ECG, CWR test (including breath-by-breath cardiometabolic measurements and respiratory muscle EMG), quality of life (QoL) assessments (St. George's respiratory questionnaire for COPD patients [SGRQ-C] and short form-36 [SF-36]), physical activity assessments (7-day recall online 12-item questionnaire), and a SPPB to establish period baseline measurements, and will be randomly assigned to 1 of 2 treatment sequences in a 1:1 ratio.

To achieve steady-state exposure to CK-2127107, subjects will receive CK-2127107 (500 mg) or matching placebo twice daily for 14 days, except on day 1 and day 14. On day 1, subjects will take active or placebo drug once in the evening, and on day 14, subjects will take active or placebo drug once in the morning. At visit 5, subjects will also have renal and liver safety assessments.

The subjects will then be invited back to the study site again on day 14 (visit 6) for the main assessment day (MAD) visit for treatment period 1. Subsequent visits for the second period baseline (visit 7) and treatment period 2 will then follow as per the Schedule of Assessments [Table 1].

During the study, blood samples will also be collected at predefined time points for the assessment of plasma concentrations of CK-2127107 and possible metabolite(s) (if applicable) [See Section 5.6]. One (voluntary) blood sample for pharmacogenomics will also be taken prior to first dose at day 1 of treatment period 1 (predose).

For all subjects, safety will be assessed through the monitoring of AEs concomitant medications, vital signs (including blood pressure, heart rate and body temperature), clinical laboratory assessments, physical examination and ECG parameters.

Upon completion of 2 treatment periods or ED, each subject will attend a FUV (visit 10), 7 to 14 days after the last dose or early withdrawal.

An overview and planning of all assessments can be found in the Flow Chart [Figure 1] and Schedule of Assessments [Table 1].

2.2.2 Dose Rationale

The optimal dose for CK-2127107 has not yet been determined, but CK-2127107 is expected to improve the physical (skeletal muscle) function in subjects at the same dose range and plasma concentration as those in healthy subjects (Study CY 5013). The nonclinical studies conducted in human diaphragm obtained from biopsies indicated that CK-2127107 restored the contractile force of muscle fibers from subjects on mechanical ventilation to that of control muscle fibers. In an assessment of running performance (NCD11-055), CK-2127107 increased running time in healthy rats and rats with heart failure under slowly accelerating conditions on a rotarod apparatus.

In the CY 5013 pharmacokinetic-pharmacodynamic study, the change in the force-frequency profile of the tibialis anterior muscle during transcutaneous stimulation of the common peroneal nerve and its relationship to CK-2127107 dose and plasma concentrations CK-2127107 was evaluated in healthy male subjects. CK-2127107 (300 and 1000 mg single dose) was associated with statistically significantly greater changes in the force generated by the tibialis anterior muscle from baseline to all time points evaluated (1, 3, 5 and 7 hours postdose) versus placebo. The mean increase in force from baseline relative to placebo in CK-2127107 plasma concentration range > 1000 to 2000 ng/mL was 31.57%. Thus, maintaining CK-2127107 plasma concentration in the range of 1000 to 2000 ng/mL or higher is expected to produce a pharmacodynamic effect with CK-2127107 in humans. The 500 mg twice daily (daily dose of 1000 mg) was chosen based on the results of study CY 5013.

The results of the CY 5012 multiple ascending dose study, demonstrated that steady-state CK-2127107 mean plasma C_{trough} of 500 mg twice daily was higher than 2000 ng/mL, and was therefore considered an appropriate dose to be explored in a proof of pharmacology study while maintaining plasma exposures proven to be efficacious in the pharmacokinetic-pharmacodynamic study (CY 5013).

2.3 Endpoints

2.3.1 Primary Endpoints

The primary endpoint is the change from period baseline in CWR endurance time relative to placebo. The CWR defines how long it takes until the subject reaches symptom limitations while simultaneously being monitored and is called “CWR time to intolerance” which determines the “CWR endurance time”.

CWR endurance time will be performed during screening and on day 1 and day 14 during each of the 2 treatment periods or at early discontinuation (ED) [Table 1].

2.3.2 Secondary Endpoints

The secondary endpoints are as follows:

- Change from period baseline in VO_2, $V_{E,}$ V_E/VCO_2, IC, as well as other cardiometabolic variables and perceived exertion for dyspnea and leg discomfort (Borg CR10) at isotime* and peak during CWR as compared to placebo

- Change from period baseline in activation of accessory respiratory muscles (by EMG) at isotime* and peak exercise during CWR
- Change in resting spirometry compared to placebo

*Isotime is the shortest duration of all of the CWR tests performed before and after treatment, not including screening.

These endpoints relate to the isotime physiologic measurements that underpin the increase in exercise tolerance and represent an integrated examination of cardiopulmonary measurements. The analysis of variables that are recorded breath-by-breath (e.g., VO_2, V_E, V_E/VCO_2) are facilitated by averaging data over 20 second intervals within the metabolic cart system (recorded by the Vmax breath-by-breath metabolic measurement system). Surface accessory muscle EMG activity is recorded throughout the exercise and analyzed over the same time intervals as the breath-by-breath data. IC is recorded every 2 minutes during exercise; its measurement requires the subject to produce a maximum inspiration (from end-inspiration) on command every 2 minutes during the exercise test. This allows the determination of whether IC is reduced during exercise as a consequence of dynamic hyperinflation, which is common during exercise in COPD. The exercise system software stores this maneuver and facilitates post-test examination of each maneuver to assess quality. Therefore IC assessment of dynamic hyperinflation is obtained with 2-minute resolution.

Resting spirometry is measured before exercise but after inhalation of albuterol. It is measured at approximately the same time of day, at the same duration after the subjects' morning dose of normal bronchodilator medications. Subjects will receive 2 puffs of albuterol in the laboratory 10 to 15 minutes prior to the spirometry assessment on each study day.

2.3.3 Exploratory Endpoints

The exploratory endpoints are as follows:

- Change from period baseline in QoL outcomes (SGRQ-C, SF-36) relative to placebo
- Change from baseline in physical activity using a 7-day recall online 12-item questionnaire.

  This will be assessed during only the first treatment period. Therefore, this will represent the response to placebo for half the subjects and the response to the study drug for the other half of subjects.
- Change from period baseline in physical performance tests battery score (SPPB) relative to placebo
- Change from period baseline in MIP and MEP relative to placebo. This test is performed before exercise testing and is part of the resting assessment.

2.3.4 Safety Endpoints

- AEs/SAEs
- Vital signs (blood pressure, heart rate, body temperature)
- Clinical laboratory tests (serum chemistry, hematology and urinalysis)
- 12-lead ECG

2.3.5 Pharmacokinetic Endpoints

- Pharmacokinetics of CK-2127107 and possible metabolite(s) (if applicable):
  - Day 1 (only treatment period 2): predose plasma concentration
  - Day 14 (of both treatment period 1 and 2): C_{trough} and C_{6h}

3 STUDY POPULATION

3.1 Selection of Study Population

This study will randomize subjects with a clinical diagnosis of moderate to severe COPD between 40 to 75 years of age inclusive. Approximately 40 subjects are planned to be randomized to 1 of 2 treatment sequences in a crossover fashion. Enrollment for this study will be at approximately 4 sites in the United States and possibly Canada or the United Kingdom.

The inclusion/exclusion criteria are to screen for the appropriate subjects to enroll in this study. If a subject fails to meet these parameters the subject will not be included in the study.

3.2 Inclusion Criteria

A subject is eligible for the study if all of the following apply:

1. Institutional review board (IRB)-/independent ethics committee (IEC)-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Male or female aged 40 to 75 inclusive, at the signing of the informed consent.
3. Subject has a body mass index (BMI) of 18 to 35 kg/m^2 inclusive.
4. Subject must have all of the following:
   a. Clinical diagnosis of moderate to severe COPD, with a postbronchodilator FEV_1/FVC ratio < 70% and 30% ≤ FEV_1 < 80% predicted at screening. The predicted values for normal spirometry will be those recommended by the ATS/ERS [Miller et al, 2005].
   b. General stable health with no change in medication (including non-COPD agents and dietary aids/food supplements) within 2 weeks prior to screening, no systemic corticosteroid administration (topical or inhaled corticosteroids are allowed) within 6 weeks prior to screening, no exacerbations or hospitalization within 6 weeks prior to screening.
   c. Current or ex-smokers with a smoking history of at least 10 pack years*.

d. Grade of 2 or 3 on the Modified Medical Research Council (mMRC) Dyspnea Scale [Appendix 12.8] at screening:
   - Grade 2: walks slower than people of the same age on the level because of breathlessness or has to stop for breath when walking at own pace on the level
   - Grade 3: stops for breath after walking about 100 meters or after a few minutes on the level

5. Subject is able to complete technically acceptable respiratory muscle strength tests, spirometry, physical performance test, and exercise tests.
6. This criterion has been deleted.
7. Female subject must either:
   - Be of non-child bearing potential:
     - Postmenopausal (defined as at least 1 year without any menses) prior to screening, or
     - Documented surgically sterile
   - Or, if of childbearing potential,
     - Agree not to try to become pregnant during the study and for 28 days after the last dose,
     - And have a negative serum pregnancy test at screening,
     - And, if heterosexually active, agree to consistently use 2 forms of highly-effective birth control** (at least 1 of which must be a barrier method) starting at screening, throughout the study, and for 28 days after the last dose.
8. Female subject must agree not to breastfeed starting at screening and throughout the study and for 28 days after the last dose.
9. Female subject must not donate ova starting at screening, throughout the study and for 28 days after the last dose.
10. Male subject and their female spouse/partners who are of childbearing potential must be using highly effective form of contraception consisting of 2 forms of birth control** (at least 1 of which must be a barrier method) starting at screening, and continuing throughout the study and for 90 days after the last dose.
11. Male subject must not donate sperm starting at screening, throughout the study and for 90 days after the last dose.
12. Subject agrees not to participate in another interventional study from screening through the FUV of the study.

*Pack year is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. For example, 1 pack year is equal to smoking 1 pack per day for 1 year, or 2 packs per day for half a year, and so on.

**Highly effective forms of birth control include:

- Consistent and correct usage of established oral contraception
- Established intrauterine device (IUD) or intrauterine system (IUS)
- Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository

Waivers to the Inclusion Criteria will NOT be allowed.

3.3 Exclusion Criteria

Subjects will be excluded from participation if any of the following apply:

1. Subject has previously enrolled in a clinical study of CK-2127107.
2. Subject has any clinically significant abnormality following the investigator's review of the physical examination, ECG and protocol-defined clinical laboratory tests at screening. A significant abnormality is defined as an abnormality which, in the opinion of the investigator, may (i) put the subject at risk because of participation in the study, (ii) influence the results of the study, or (iii) cause concern regarding the subject's ability to participate in the study.
3. Subject has any of the LFTs (i.e., AST, ALT, ALP, γ-glutamyl transferase [GGT] and/or TBL) above 1.5 times the ULN at screening. These assessments may be repeated once at the investigator's discretion (within the screening window).
4. Subject has an estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m^2 by the Cockcroft-Gault equation at screening.
5. Subject has a serious cardiovascular disease, including a current New York Heart Association (NYHA) class III or IV congestive heart failure or clinically significant valvular disease, history of cardiac arrest, uncontrolled angina or arrhythmia, untreated serious conduction disorder (e.g., third-degree heart block), or acute myocardial ischemic condition suspected on the ECG at screening (e.g., ST-segment elevation, ST-segment depressions > 2 mm).
6. Subject has had a myocardial infarction or other acute coronary syndrome, major heart surgery (i.e., valve replacement or bypass surgery), stroke, deep vein thrombosis or pulmonary embolus in the 6 months prior to screening.
7. Subject has known active tuberculosis.
8. Subject has undergone thoracotomy with pulmonary resection (except for sub-lobar resection).
9. Subject has resting pulse < 40 bpm or > 100 bpm; resting systolic blood pressure > 160 mm Hg or < 90 mm Hg; resting diastolic blood pressure > 100 mm Hg at screening. These assessments may be repeated once at the investigator's discretion (within the screening window).
10. Subject desaturates to SpO_2 < 85% for at least 1 minute on screening IET.

11. Subject has a limitation of exercise performance as a result of factors other than fatigue or exertional dyspnea/shortness of breath (considered to be due to COPD), such as arthritis in the leg, angina pectoris, heart failure, claudication or morbid obesity.
12. Subject has a CWR cycle ergometry endurance time less than 4 or greater than 8 minutes after WR adjustment procedures at screening visit 2.
13. Subject has used the following drugs within 14 days prior to day -1:
    a. Strong CYP3A4 inhibitor (e.g., itraconazole, clarithromycin).
    b. Strong CYP3A4 inducer (e.g., barbiturates, rifampin).
14. Subject has hemoglobin (Hb) concentration below 10.0 g/dL at screening.
15. Subject has a cancer requiring treatment currently or in the past 3 years (except primary non-melanoma skin cancer, carcinoma in situ or cancers that have an excellent prognosis such as early stage breast or prostate cancer).
16. Subject giving a history of asthma, allergic rhinitis or atopy shall be evaluated by the investigator to determine whether the subject's predominant diagnosis is COPD rather than asthma.
17. Subject has neurological conditions or neuromuscular diseases that are causing impaired muscle function or mobility.
18. Subject has a current diagnosis of schizophrenia, other psychotic disorders or bipolar disorder.
19. Subject in the active phase of pulmonary rehabilitation or had completed pulmonary rehabilitation or exercise training within the 13 weeks prior to screening.
20. Subject has severe and/or uncontrolled medical conditions that could interfere with the study (e.g., severe neurological deficit after stroke, developed diabetic peripheral neuropathy, respiratory diseases requiring daytime supplemental oxygen, infection, gastrointestinal disorder, uncontrolled pain or any other non-stable illness) as judged by the medical investigator.
21. Subject has a known history of positive test for hepatitis B surface antigen (HBsAg) or hepatitis C antibody or history of a positive test for human immunodeficiency virus (HIV) infection.
22. Subject has a history of alcoholism or drug/chemical substance abuse within 2 years prior to screening.
23. Subject has used any medications known to affect physical function or muscle mass including androgen supplements, anti-androgens (such as luteinizing hormone-releasing hormone [LHRH] agonists), anti-estrogen (tamoxifen, etc.), recombinant human growth hormone (rhGH), oral beta adrenergic agonists, megestrol acetate, dronabinol, or other drugs which, in the opinion of the investigator, might influence physical function or muscle mass within 6 weeks prior to screening.

24. Subject has participated in any interventional clinical study or has been treated with any investigational drugs within 28 days or 5 half-lives whichever is longer, prior to the initiation of screening.
25. Subject has any other condition that in the opinion of the investigator precludes the subject's participation in the trial.

Waivers to the Exclusion Criteria will NOT be allowed.

4 TREATMENT(S)

4.1 Identification of Investigational Product(s)

4.1.1 Study Drug(s)

CK-2127107 study drug will be supplied in a blinded form by Astellas as CK-2127107-C 250 mg tablets, film-coated. The tablets are white oral tablets.

4.1.2 Comparative Drug(s)

Placebo for CK-2127107-C tablets will be supplied by Astellas in a blinded form to match the active tablets.

4.1.3 Rescue Drug(s)

In the event of a bronchospasm, albuterol is allowed to be self-administered by the subject, except for testing days (visits 4, 6, 7 and 9). Subjects requiring rescue albuterol on testing days (beyond the dose administered with spirometry) before completing all assessments will return to the clinic as soon as possible to repeat the entire study day.

NOTE: During the scheduled spirometry assessments the subjects will receive 2 puffs of albuterol in the laboratory 10 to 15 minutes prior to the spirometry assessment on each study day. Requirement of additional "rescue" doses would indicate that that the subject's disease is not stable.

The allowable dose and mode of administration for albuterol is 180 µg (2 puffs): inhaled by mouth every 4 to 6 hours; not to exceed 12 inhalations/24 hours.

4.2 Packaging and Labeling

All study drugs used in this study will be prepared, packaged, and labeled under the responsibility of qualified staff at Astellas or Sponsor's designee in accordance with Astellas or Sponsor's designee standard operating procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, and applicable local laws/regulations.

Each bottle, containing 30 tablets of CK-2127107 or matching placebo, will bear a label conforming to regulatory guidelines, GMP and local laws and regulations which identifies the contents as investigational drug.

CK-2127107 and matching placebo should be stored in the original bottle at United States Pharmacopeia controlled room temperature, 20°C to 25°C (68°F to 77°F) with excursions permitted from 15°C to 30°C (59°F to 86°F).

4.3 Study Drug Handling

Current ICH GCP Guidelines require the investigator to ensure that study drug deliveries from the Sponsor are received by the investigator/or designee and that such deliveries are recorded, that study drug is handled and stored according to labeled storage conditions, that study drug with appropriate expiry/retest is only dispensed to study subjects in accordance with the protocol, and that any unused study drug is returned to the Sponsor.

Drug inventory and accountability records for the study drugs will be kept by the investigator or designee. Study drug accountability throughout the study must be documented and reconciled. The following guidelines are therefore pertinent:

- The investigator agrees not to supply study drugs to any persons except the eligible subjects in this study in accordance with the protocol.
- The investigator or designee will keep the study drugs in a pharmacy or other locked and secure storage facility under controlled storage conditions, accessible only to those authorized by the investigator to dispense these study drugs.
- A study drug inventory will be maintained by the investigator or designee. The inventory will include details of material received and a clear record of when they were dispensed and to which subject.
- At the conclusion or termination of this study, the investigator or designee agrees to conduct a final drug supply inventory and to record the results of this inventory on the Drug Accountability Record. It must be possible to reconcile delivery records with those of used and/or returned medication. Any discrepancies must be accounted for and documented. Appropriate forms of deliveries and returns must be signed by the site staff delegated this responsibility.
- The site must return study drug to the Sponsor or designee at the end of the study or upon expiration.

4.4 Blinding

4.4.1 Blinding Method

This is a double blind study. Subjects will receive CK-2127107 or matching placebo in a double-blind fashion such that the investigator, Sponsor's study management team, clinical staff and the subjects will not know which agent is being administered. The randomization number will be assigned based on information obtained from the Interactive Response Technology (IRT).

4.4.2 Confirmation of the Indistinguishability of the Study Drugs

The appearance, size and color of the CK-2127107-C 250 mg tablets, film coated are the same as the matching placebo.

4.4.3 Retention of the Assignment Schedule and Procedures for Treatment Code Breaking

The randomization list and study drug blind will be maintained by the IRT system. A separate instruction manual will be provided to the investigator and staff on use of the IRT for randomization and generation of randomization number.

4.4.4 Breaking the Treatment Code for Emergency

In the event of a medical emergency requiring knowledge of a subject's treatment assignment, only the investigator or other persons designated as subinvestigators are authorized to receive a subject's treatment assignment after approval by the Astellas Medical Monitor or Astellas Pharmacovigilance (PSP) physician. For immediate access to treatment code assignments, the IRT system provides a 24-hour telephone Global Help Desk service to assist users 365 days a year. In the event of a problem accessing the IRT system or completing a transaction, the IRT Help Desk will provide assistance.

If the blind is broken, the time, date, subject number, and reason for breaking the blind must be documented. Subjects and other study personnel will not be made aware of unblinding unless a medical emergency necessitates such disclosure. The subject numbers of unblinded subjects will be reported to the Sponsor before database lock.

4.4.5 Breaking the Treatment Code by the Sponsor

The Sponsor may break the treatment code for subjects who experience a suspected unexpected serious adverse reaction (SUSAR), in order to determine if the individual case or a group of cases requires expedited regulatory reporting. Individual Emergency Codes will be provided to the limited staff who are responsible to break the codes for all SUSAR cases for reporting purposes.

The treatment code for each randomized subject will be provided by the IRT in the event of a medical emergency requiring knowledge of the treatment assigned to the subject. The time, date, subject number and reason for obtaining any of these codes, and therefore breaking the blind, must be documented in the study file. They must only be requested by the investigator or other persons designated as subinvestigators. No subjects or other study personnel will be made aware of the treatment given to any subject unless a medical emergency necessitates such disclosure.

Unblinding of the study drug should only be considered for subject safety or when critical therapeutic decisions are contingent upon knowing the blinded study drug assignment. Any unblinding by the investigational staff must be reported immediately to the Sponsor and must include an explanation of why the study drug was unblinded. If possible, the Sponsor should be contacted prior to unblinding of the study drug.

4.5 Assignment and Allocation

Randomization will be performed via IRT. Prior to the initiation of the study treatment, the site staff will contact the IRT in order to determine the randomly assigned treatment sequence. Specific procedures for randomization through the IRT are contained in the study procedures manual.

5 TREATMENTS AND EVALUATION

5.1 Dosing and Administration of Study Drug(s) and Other Medication(s)

5.1.1 Dose/Dose Regimen and Administration Period

Subjects will be screened up to 28 days prior to randomization. Informed consent/ authorization will be obtained prior to randomization and before any study-related procedures are performed.

Subjects will be randomly assigned to 1 of 2 treatment sequences and will receive CK-2127107 as two 250 mg tablets (500 mg twice a daily [1000 mg daily total]) or 2 tablets twice daily of matching placebo during the 2 treatment periods. Doses are to be taken in the morning and evening within 2 hours after a meal (approximately 12 hours apart). Each treatment period consists of 14 days. Each medication period is separated by a 14-day washout period.

The time of daily study drug dosing is to be recorded in the paper diary. Subjects will take study drugs as follows: once a day after dinner at day 1, twice a day within 2 hours after breakfast and dinner between day 2 and day 13 and once a day within 2 hours after breakfast at day 14. At day 14, in order to collect the trough pharmacokinetic sample, the subject is to wait and take the morning dose at the site after pharmacokinetic sample collection has been performed.

5.1.2 Increase or Reduction in Dose of the Study Drug(s)

No increase or reduction of CK-21271070 will be allowed for an individual subject.

5.1.3 Previous and Concomitant Treatment (Medication and Non-Medication Therapy)

Concomitant Medication/Food Restrictions or Requirements:

Change in medication (including non-COPD agents and dietary aids/food supplements) will not be allowed within 2 weeks prior to screening.

No systemic corticosteroid administration (except for topical or inhaled corticosteroids) is allowed within 6 weeks prior to screening.

The following concomitant medications and treatments are prohibited as described:

- Systemic corticosteroids
- Strong CYP3A4 inhibitors (e.g., itraconazole, clarithromycin, grapefruit juice, Seville oranges) [see Appendix 12.1]

- CYP3A4 inducers (e.g., barbiturates, rifampin, St. John's wort) [see Appendix 12.1]
- Cannabis/tetrahydrocannabinol (THC)-based medication
- Skeletal muscle relaxants
- OCT1/OCT2 substrates: oxaliplatin, pindolol, varencicline, pilisicainide and dofetilide
- Any medications known to affect physical function or muscle mass including androgen supplements, anti-androgens (such as LHRH agonists), anti-estrogen (tamoxifen, etc.), rhGH, oral beta adrenergic agonists, megestrol acetate, dronabinol, or other drugs which, in the opinion of the investigator, might influence physical function or muscle mass [Appendix 12.1].

Caution is recommended for concomitant administration of the OCT1 substrates (e.g., aciclovir, ganciclovir) and OCT1/2 substrates (e.g., in particular metformin [antidiabetic], dofetilide, pindolol) as CK-2127107 may have the potential to inhibit OCT1 and OCT2-mediated transport [Appendix 12.2].

In case non-permitted treatment is necessary, withdrawal of the subject from the study should be considered by the authorized medical personnel.

The following **will** be allowed during the study:

- Chronic medications including COPD and non-COPD agents and dietary aids/food supplements are permitted, but change in the dose regimen or dosage, or starting any new medication is not allowed after the screening evaluations. The subject should be in a stable state, including prescribed medications throughout the study.
- Albuterol (180 µg) is to be administered by inhalation 10 to 15 minutes prior to spirometry assessment on each study day.

All concomitant medication usage will be noted and recorded during each study visit.

A list of excluded concomitant medication is provided in [Appendix 12.1].

5.1.4 Treatment Compliance

Study subjects should be counseled on the need to meet 100% compliance with study drug. Investigator or designee should ensure that study subjects meet this goal throughout the study period. Compliance will be verified by the accounting of study drug as recorded by the subject via paper diary. Compliance of the study drug will be monitored by the accounting of unused medication returned by the subject at visits. When study drug is administered at the research facility, it will be administered under the supervision of study personnel.

Treatment compliance should be monitored closely, and deviation in treatment compliance, such as receiving the wrong treatment or dose, should be reported to the Sponsor; the appropriate documents should be submitted and, if applicable, reported to the IRB/IEC.

5.2 Demographics and Baseline Characteristics

5.2.1 Demographics

Demographic and baseline characteristics will be collected during screening for all subjects according to the Schedule of Assessments [Table 1] and will include date of birth, sex, race and ethnicity, height, body weight, smoking history and alcohol use. Vital signs such as heart rate, blood pressure, body temperature as well as resting ECG are measured during screening. Heart rate and ECG will also be recorded simultaneously during all IET and CWR exercise tests. Blood pressure will be taken by auscultation during exercise tests periodically (approximately every 2 min) and recorded in the electronic exercise test record.

At screening a full pulmonary function test that includes force expiratory flows, lung volumes (including total lung capacity [TLC], IC and residual volume [RV]) and diffusing capacity will be performed. This will characterize the subject's lung function and disease for entry into the study. MIP and MEP are measured following the resting spirometry test prior to the exercise test (visits 4, 6, 7 and EOT only). During the remainder of the trial, only the spirometry (FEV, FVC) and MIP/MEP will be measured. Spirometry (FEV_1, FEV_1/FVC, etc.) will be performed according to ATS guidelines [Miller et al, 2005].

5.2.2 Medical History

Site personnel will collect and record information regarding the subject's medical history at screening and any updated information will be recorded on study day 1 of each treatment period.

Medical history will include: subject diagnosis for clinical diagnosis of moderate to severe COPD, with a postbronchodilator $FEV_1/FVC < 70\%$ and $30\% \leq FEV_1 < 80\%$ predicted at screening. Medical records will be reviewed whenever possible. In cases where the investigator decides that COPD is the predominant diagnosis (and that the subject is to be included), his/her reasoning shall be explained in a note to the subject's study file.

Additionally, the following information will be obtained: BMI, grade on the mMRC Dyspnea Scale at screening and smoking history.

5.2.3 Diagnosis of the Target Disease, Severity and Duration of Disease

Subjects must have clinical diagnosis of moderate to severe COPD, with a postbronchodilator $FEV_1/FVC < 70\%$ and $30\% \leq FEV_1 < 80\%$ predicted at screening; general stable health with no change in medication (including non-COPD agents) and no exacerbations or hospitalization within 6 weeks prior to screening; and grade of ≥ 2 on the mMRC Dyspnea Scale [Appendix 12.8] at screening.

5.3 Efficacy | Exploratory | Pharmacokinetics Assessment

5.3.1 Efficacy Assessment

Refer to the Study Procedure Manual for details regarding the individual assessments for testing subject endurance. The efficacy assessments will be performed per the Schedule of Assessments [Table 1].

5.3.1.1 IET and CWR

Subjects will perform IET and a CWR exercise test on a cycle ergometer. IET will be performed at screening visit 1 and characterizes the subject and determines the WR to use for CWR exercise test. The IET will be performed only once and characterizes the subject's aerobic capacity (VO_2 peak), lactate threshold, ventilatory efficiency (V_E/VCO_2 nadir) and peak WR. The IET will not be repeated. At screening visit 2, the CWR test will be completed at least 24 hours after the IET. Additionally the CWR test will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9).

In this protocol, a Vmax breath-by-breath metabolic measurement system with integrated 12-lead ECG and cycle ergometer will be used. All variables are recorded breath-by-breath in the cardiopulmonary exercise testing system. Blood pressure, IC, as well as Borg CR10 score ratings of dyspnea and leg discomfort, are recorded at isotime and peak during the CWR test. Blood pressure is measured manually by auscultation. These values are manually entered into the electronic Vmax record of in the metabolic system during the test.

The ergometer is computer controlled by the metabolic system and delivers the desired WR profile (IET or CWR). The ECG is monitored continuously; heart rate is derived beat-by-beat from the ECG. A 12-lead configuration for the initial IET will be used, so that a full diagnostic recording can be made. In CWR tests, a 3-lead configuration will be used. The ECG is stored in the integrated software program, which facilitates analyses such as arrhythmias and ST segment changes. A test's ECG can be stored in full (the full beat-by-beat record) or a summary form. Heart rate is recorded to the breath-by-breath record in the Vmax breath-by-breath metabolic measurement system. Generally, the subject will have about 5 minutes of recovery recorded (i.e., with mouthpiece in place and ECG being recorded).

The selection of the work rate for the initial testing has to be individualized to the subject's exercise tolerance [Casaburi R, 2005]. Establishing a period baseline duration for all subjects (normalized to within the 4 to 8 minute limit) allows for the correct interpretation of the change in post-treatment intervention exercise duration among different individuals' ERS Statement [Puente-Maestu et al, 2016].

CWR time to intolerance (endurance time) is assessed by a stopwatch. This is verified from the electronic record of the power output on the cycle ergometer.

5.3.1.2 EMG

EMG will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9). Surface accessory muscle EMG activity will be measured during the exercise test. Surface electrodes are applied during the set-up for the exercise test; their application takes 10 minutes. The continuous time course of the EMG from each electrode is recorded for subsequent analysis.

5.3.1.3 Borg CR10

Borg CR10 will be conducted during screening (visit 2). Additionally Borg CR10 will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9).

The 10-item Borg Scale [Borg et al, 1982] is a simple method of rating perceived exertion (RPE) and collects information on perceived exertion in an individual's rating of exercise intensity [Appendix 12.9]. The Borg CR10 will be administered and scored by the investigator or delegated research staff. Subjects will be asked to use this scale to rate the intensity of their breathing and leg discomfort before, during, and after exercise.

5.3.1.4 Physical Activity

Physical activity will be measured by an online 12-item PRO questionnaire (7-day recall [C-PPAC]) which has to be completed at the site. Physical activity is assessed by questions targeted at the amount of physical activity and perceived difficulty during physical activity.

The European Union Innovative Medicines Initiative PROactive project [Gimeno-Santos et al, 2015] conducted qualitative research and drafted a conceptual framework to set out the concept of physical activity from patients' experiences and to provide the necessary basis to generate an item pool for 2 PROs, 1 with a daily recall period and 1 with a 7-day recall period (daily and clinical visit versions, respectively) to measure the experience of physical activity in patients with COPD.

5.3.1.5 Spirometry

Spirometry will be performed at screening visit 1, treatment period 1 (visits 4 and 6), treatment period 2 (visits 7 and 9) and FUV (visit 10). Spirometry measures the volume of air exhaled at specific time points during a forceful and complete exhalation after a maximal inhalation (i.e., the total exhaled volume, known as FVC, the volume exhaled in the first second, known as FEV_1, and their ratio [FEV_1/FVC] are measured). Spirometry will be performed according to ATS guidelines. Resting spirometry is measured before exercise but after inhalation of albuterol. It is measured at approximately the same time of day, at the same duration after the subject's morning dose of normal bronchodilator medications. This is also to confirm whether the drug affects pulmonary function in subjects. Also, a marked variation (especially a worsening) would be a sign that the subject is not in a stable state or may be suffering an exacerbation.

5.3.2 Exploratory Assessment

5.3.2.1 MIP and MEP

Respiratory muscle strength tests, MIP and MEP will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9). MIP is the maximum negative pressure that can be generated from 1 inspiratory effort starting from functional residual capacity (FRC) or RV. In this study, the test will be performed from a maximal exhalation to RV. MEP measures the maximum positive pressure that can be generated from 1 expiratory effort starting from TLC. Unlike inspiratory muscles, expiratory muscles (abdominal and thoracic muscles) reach their optimal force–length relationship at large pulmonary volumes. MIP reflects the

strength of the diaphragm and other inspiratory muscles, while the MEP reflects the strength of the abdominal muscles and other expiratory muscles as well as lung and chest wall elastic rebound.

5.3.2.2 SGRQ-C

The SGRQ-C [Jones PW et al, 1991; Jones PW et al, 1992; Merguro M et al, 2007] will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9). The SGRQ-C was developed from the SGRQ which was designed to measure health impairment in patients with asthma and COPD. The SGRQ-C was developed using COPD data only, and this version is shorter with 40 items (14 questions) compared to the original 50 items. The questionnaire is designed for supervised self-administration, site staff will be available to ensure the subject completes the questionnaire. The questionnaire will be administered electronically. The SGRQ-C will be scored by Astellas using an excel-based scoring system provided by St. George's University of London.

5.3.2.3 SF-36

The SF-36 will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9). The questionnaire will be self administered by subject electronically at the site. SF-36 was designed for use in clinical practice and research, health policy evaluations and general population surveys.

The SF-36 is a QoL instrument designed to assess generic health concepts relevant across age, disease, and treatment groups. It is aimed at both adults and adolescents aged 18 years and older. It has been used in a range of diseases (non-disease specific).

The SF-36 consists of 8 domains of health status: Physical functioning (10 items), Role-physical (4 items), Bodily pain (2 items), General health (5 items), Vitality (4 items), Social functioning (2 items), Role emotional (3 items) and Mental health (5 items). Two component scores, the Physical Component Summary and the Mental Component Summary can also be calculated.

For both the SF-36 domain scores and summary scores, higher scores indicate better health status. The SF-36 has a recall period of the 'past 4 weeks'.

The SF-36 will be scored by quality metrics.

5.3.2.4 SPPB

SPPB will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9). SPPB is a simple test to measure lower extremity function using tasks that mimic daily activities; static balance, gait speed and getting in/out of a chair.

The SPPB score is based on timed measures of standing balance, walking speed and ability to rise from a chair. For the balance test, subjects will be asked to maintain their feet in side-by-side, semi-tandem (heel of 1 foot beside the big toe of the other foot) and tandem (heel of 1 foot in front and touching the other foot) positions for 10 seconds each. Walking speed will be assessed by asking subjects to walk at their usual pace over a 4-meter course. Two

walk times will be recorded, and the faster of the 2 will be used to compute the walking test score. For the chair test, subjects will be asked to stand up from a sitting position with their arms folded across the chest. If subjects are able to perform this task, they will be asked to stand up and sit down 5 times as quickly as possible, and the time to perform the test will be recorded. Each of the 3 performance measures will be assigned a score ranging from 0 to 4, with 4 indicating the highest level of performance and 0 the inability to complete the test. A summary score (range 0 to 12) will be subsequently calculated by adding the 3 scores. For the test of balance, subjects will be assigned the following scores: 1 if they could only hold a side-by-side standing position for 10 seconds; 2 if they could hold a semi-tandem position for 10 seconds, but were unable to hold a full-tandem position for more than 2 seconds; 3 if they could stand in a full-tandem position for 3 to 9 seconds; and 4 if they could stand in a full-tandem position for 10 seconds [Guralnik et al, 1994].

5.3.3 Pharmacokinetics Assessment

Blood samples to assess the pharmacokinetics of CK-2127107 and possible metabolite(s) (if applicable) are to be predose collected on day 1 of treatment period 2, day 14 and end of treatment (EOT) and 6 hours postdose on day 14 and EOT.

5.4 Safety Assessment

The safety assessments include the following and will be performed per the Schedule of Assessments [Table 1]:

1. AEs/SAEs are to be assessed at every visit.
2. A 12-lead ECG will be performed at screening and at visits 4, 6, 7, 9 and 10.
3. The following clinical laboratory tests will be collected at screening and visits 4, 5, 6, 7, 8, 9 and 10:
    - Serum chemistry
    - Hematology (excluding visits 5 and 8)
    - Urinalysis (excluding visits 5 and 8)
4. A standard urine drug screen and alcohol screen will be performed at screening and day 1 of each treatment period.

5.4.1 Vital Signs

Vital signs include blood pressure, heart rate and body temperature.

5.4.2 Adverse Events

See [Section 5.5] for information regarding AE collection and data handling.

5.4.2.1 Adverse Events of Possible Hepatic Origin

See [Appendix 12.3] for detailed information on liver abnormalities, monitoring and assessment, if the AE for a subject enrolled in a study and receiving study drug is accompanied by increases in liver function tests (LFTs; e.g., AST, ALT, bilirubin, etc.) or is suspected to be due to hepatic dysfunction.

Subjects with AEs of hepatic origin accompanied by LFT abnormalities should be carefully monitored.

5.4.2.2 Adverse Events of Possible Renal Origin

See [Appendix 12.4] for details. Subjects shall be discontinued from the study drug should their serum creatinine demonstrate an increase of 1.5 to 2.0 times above baseline (CTCAE grade 1) in concordance with an increase in serum cystatin C levels.

5.4.3 Laboratory Assessments

[Appendix 12.6] lists the laboratory tests that will be performed during the conduct of the study. See the Schedule of Assessments [Table 1] for study visit collection dates.

Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator/subinvestigator who is a qualified physician.

5.4.4 Physical Examination

A full physical examination will be performed at screening visit 1 and follow-up visit 10. Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal, and neuromuscular. At the visits 4, 6, 7, and 9, a symptom-directed physical exam will be performed.

Any clinically relevant abnormality at screening will be recorded as medical history. Any clinically relevant change from baseline to visit 10/FUV will be recorded as an AE in the eCRF. Documentation that an exam has occurred needs to be reflected in the site source documents.

Screening physical examination is to be conducted in person by a physician. Remaining physical examinations can be symptom-directed and delegated to a Nurse Practitioner or Physician Assistant (if the study physician is unavailable) and signed off by the study physician.

5.4.5 Electrocardiogram (ECG)

A standard 12-lead ECG will be conducted at screening and on visits 4, 6, 7, 9 and 10. Specific attention will be given to the ST segment at screening to assess eligibility for this study. The 12-lead ECGs will be recorded in triplicate (3 separate ECGs) and transmitted electronically for central reading.

5.5 Adverse Events and Other Safety Aspects

5.5.1 Definition of Adverse Events (AEs)

An AE is defined as any untoward medical occurrence in a subject administered a study drug or has undergone study procedures and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Some countries may have additional local requirements for events that are required to be reported as AEs or in an expedited manner similar to an SAE. In these cases, it is the investigator's responsibility to ensure these AEs or other reporting requirements are followed and the information is appropriately recorded in the electronic case report form (eCRF) accordingly.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical examination) should be defined as an AE only if the abnormality meets 1 of the following criteria:

- Induces clinical signs or symptoms
- Requires active intervention
- Requires interruption or discontinuation of study drug
- The abnormality or test value is clinically significant in the opinion of the investigator.

5.5.2 Definition of Serious Adverse Events (SAEs)

An AE is considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- Results in death
- Is life threatening (an AE is considered "life-threatening" if, in the view of either the investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death).
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly or birth defect
- Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious)
- Other medically important events

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent 1 of the other outcomes listed in the definition above. These events, including those that may result in disability/incapacity, should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Safety events of interest on the medicinal products administered to the subject as part of the study (e.g., study drug, comparator, background therapy) that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of the medicinal product(s)
- Suspected abuse/misuse of the medicinal product(s)

- Inadvertent or accidental exposure to the medicinal product(s)
- Medication error involving the medicinal product(s) (with or without subject exposure to the Sponsor medicinal product [e.g., name confusion])

All of the events of interest noted above should be recorded on the eCRF. Any situation involving these events of interest that also meets the criteria for an SAE should be recorded on the AE page of the eCRF and marked 'serious' on the SAE worksheet.

The Sponsor has a list of events that they classify as "always serious" events. If an AE is reported that is considered to be an event per this classification as "always serious," additional information on the event may be requested.

5.5.3 Criteria for Causal Relationship to the Study Drug

AEs that fall under either "Possible" or "Probable" should be defined as "AEs whose relationship to the study drugs could not be ruled out".

Causal relationship to the study drug	Criteria for causal relationship
Not Related	A clinical event, including laboratory test abnormality, with a temporal relationship to study drug administration which makes a causal relationship improbable, and/or in which other drugs, chemicals or underlying disease provide plausible explanations.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the study drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the study drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on re-administration (rechallenge) or withdrawal (dechallenge).

5.5.4 Criteria for Defining the Severity of an Adverse Event

The investigator will use the following definitions to rate the severity of each AE:

- Mild: No disruption of normal daily activities
- Moderate: Affect normal daily activities
- Severe: Inability to perform daily activities

5.5.5 Reporting of Serious Adverse Events (SAEs)

The collection of AEs and the expedited reporting of SAEs will start following receipt of the signed informed consent form (ICF) and will continue to 30 days after last dose of study drug. In the case of an SAE, the investigator must contact the Sponsor by telephone, email or fax immediately (within 24 hours of awareness).

The investigator should complete and submit an SAE Worksheet containing all information that is required by the Regulatory Authorities to the Sponsor by fax immediately (within

24 hours of awareness). If the faxing of an SAE Worksheet is not possible or is not possible within 24 hours, the local drug safety contact should be informed by phone.

For contact details, see Section II Contact Details of Key Sponsor's Personnel. Please email or fax the SAE Worksheet to:

Astellas Pharma Global Development – United States
Pharmacovigilance
Fax number: 1-888-396-3750
North America Alternate Fax: 1-847-317-1241
International Fax Number: +44-800-471-5263
Email: safety-us@astellas.com

If there are any questions, or if clarification is needed regarding the SAE, please contact the Sponsor's Medical Monitor/Expert or his/her designee (see Section II Contact Details of Key Sponsor's Personnel).

Follow-up information for the event should be sent promptly (within 7 days of the initial notification).

Full details of the SAE should be recorded on the medical records and on the eCRF.

The following minimum information is required:

- International Study Number (ISN)/Study number,
- Subject number, sex and age,
- The date of report,
- A description of the SAE (event, seriousness of the event), and
- Causal relationship to the study drug.

The Sponsor or Sponsor's designee will submit expedited safety reports (i.e., IND Safety Reports) to the regulatory agencies (i.e., FDA) as necessary, and will inform the investigators of such regulatory reports. Investigators must submit safety reports as required by their IRB/IEC within timelines set by regional regulations (i.e., EU, (e)CTD, FDA). Documentation of the submission to and receipt by the IRB/IEC of expedited safety reports should be retained by the site.

The Sponsor will notify all investigators responsible for ongoing clinical studies with the study drug of all SAEs which require submission per local requirements of the IRB/IEC

The investigators should provide written documentation of IRB/IEC notification for each report to the Sponsor.

Investigators and study personnel may contact the Sponsor's Medical Monitor/Expert for any other problem related to the safety, welfare, or rights of the subject.

For Suspected Unexpected Serious Adverse Reactions (SUSAR) from a blinded trial, unblinded CIOMS-I report will be submitted to the authorities and IRB/IEC where required.

5.5.6 Follow-up of Adverse Events

All AEs occurring during or after the subject has discontinued the study are to be followed up until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized.

If during AE follow-up, the AE progresses to an ″SAE″, or if a subject experiences a new SAE, the investigator must immediately report the information to the Sponsor.

Please refer to [Appendix 12.3] for detailed instructions on Drug-Induced Liver Injury (DILI).

5.5.7 Monitoring of Common Serious Adverse Events

For this protocol, there is no list of common SAEs anticipated for the study population for the purposes of IND safety reporting [Appendix 12.5].

5.5.8 Procedure in Case of Pregnancy

If a female subject or partner of a male subject becomes pregnant during the study dosing period or within 10 weeks from the discontinuation of dosing, the investigator should report the information to the Sponsor as if it is an SAE. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data etc., should be included in this information.

The investigator will follow the medical status of the mother, as well as the fetus, as if the pregnancy is an SAE and will report the outcome to the Sponsor.

When the outcome of the pregnancy falls under the criteria for SAEs (spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly [including anomaly in a miscarried fetus]), the investigator should respond in accordance with the report procedure for SAEs. Additional information regarding the outcome of a pregnancy (which is categorized as an SAE) is mentioned below.

- ″Spontaneous abortion″ includes miscarriage, abortion and missed abortion
- Death of an infant within 1 month after birth should be reported as an SAE regardless of its relationship with the study drug
- If an infant dies more than 1 month after the birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged as ″possible″ by the investigator
- In the case of a delivery of a living newborn, the ″normality″ of the infant should be evaluated at the birth
- Unless a congenital anomaly are identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination

If during the conduct of a clinical trial, a male subject makes his partner pregnant, the subject should report the pregnancy to the investigator. The investigator will report the pregnancy to the Sponsor as an SAE.

5.5.9 Emergency Procedures and Management of Overdose

No cases of overdosing have occurred thus far. In case of accidental overdose, symptom-directed supportive care is advised. It is not known whether CK-2127107 is dialyzable, and no antidote for CK-2127107 currently exists. The Medical Monitor/Expert should be contacted as applicable.

5.5.10 Supply of New Information Affecting the Conduct of the Study

When new information becomes available necessary for conducting the clinical study properly, the Sponsor will inform all investigators involved in the clinical study as well as the regulatory authorities. Investigators should inform the IRB/IEC of such information when needed.

5.6 Study Drug Concentration

Blood samples for pharmacokinetics of CK-2127107 and possible metabolite(s) (if applicable) will be collected from every subject.

A single pharmacokinetic sample will be collected at each of the following time points [Table 3].

Table 3 Pharmacokinetic Timepoints

Time window	Time point
-14 hours	Prior to evening dose at day 1 of treatment period 2
-15 min	Prior to morning dose (trough) at day 14 of both treatment period 1 and 2
±15 min	Approximately 6 hours post-morning dose at day 14 of both treatment period 1 and 2

Details on sampling, processing, storage and shipment procedures will be provided in a separate central laboratory manual. Bioanalysis of CK-2127107 and possible metabolite(s) (if applicable) will be performed using a validated liquid chromatography with tandem mass spectrometry method.

5.7 Other Measurements, Assessments or Methods

5.7.1 Blood Sample for Future Pharmacogenetic Analysis (Retrospective Pharmacogenetic Analysis)

A pharmacogenetic (PGx) research may be conducted in the future to analyze or determine genes of relevance to clinical response, pharmacokinetics and toxicity/safety issues. A PGx sample is to be collected 1 time prior to first dose at day 1 of treatment period 1 [see Table 1] a 4 mL sample of whole blood for possible retrospective PGx analysis will be collected using a vacutainer tube containing EDTA. After collection, gently invert the blood sample 8 to 10 times. The blood collection tube may either be stored upright at 4°C for up to 5 days prior to shipment or stored frozen at -20°C or below at the site for extended storage. Samples will be shipped to a Sponsor-designated banking contract research organization (CRO).

Labels should uniquely identify each sample and contain at least:

- Protocol number (3318-CL-3002),
- Subject number;
- Purpose and biological matrix (i.e., “biobanking”, “whole blood”)

Subject participation in the pharmacogenomics sample collection is optional, subjects choosing to participate will be required to sign a separate ICF.

Details on sample collection, labeling, storage and shipment procedures will be provided in the central laboratory manual.

See [Appendix 12.7] for further details on the banking procedures.

5.7.2 Exploratory Biomarker Analysis Using Metabolomics and Proteomics

Plasma samples remaining after the completion of bioanalysis of CK-2127107 and possible metabolite(s) (if applicable) may be used for an optional exploratory biomarker analysis using metabolomics and proteomics for subjects who sign a separate ICF. The purpose of the exploratory analysis is to evaluate the correlation between CK-2127107 drug response and proteomic and/or metabolome profile.

5.8 Total Amount of Blood

A total volume of approximately 216 mL of blood per subject will be collected for laboratory evaluations:

- A total volume of approximately 106 mL of blood will be collected for laboratory evaluations.
- Approximately 75 mL will be collected for safety laboratory evaluation (serum pregnancy test draws are included in this total).
- Approximately 30 mL will be collected for CK-21271017 pharmacokinetic evaluation.
- Approximately 4 mL will be collected for pharmacogenetics testing (optional).

The maximum volume of blood collected at a single visit is approximately 25 mL of blood.

6 DISCONTINUATION

6.1 Discontinuation of Individual Subject(s)

A discontinuation from treatment is a subject who enrolled in the study and for whom study treatment is permanently discontinued for any reason.

The subject is free to withdraw from the study treatment and/or the study for any reason and at any time without giving a reason for doing so and without penalty or prejudice. The investigator is also free to discontinue the subject from study treatment and to terminate a subject's involvement in the study at any time if the subject's clinical condition warrants it.

If a subject withdraws from active participation in the study (i.e., randomized and receiving study drug), the subject should be asked to complete the early discontinuation (ED) visit and return 7 days after ED visit for a FUV to assess safety and to return study drug that was provided.

This FUV should take place 7 days after the most recent study visit (ED) and follow the assessments as specified in the Schedule of Assessments [Table 1]. Study site personnel must document all assessments in the site's source documents.

If a subject is discontinued from the study with an ongoing AE or an unresolved laboratory result that is significantly outside of the reference range, the investigator will attempt to provide follow-up until the condition stabilizes or no longer is clinically significant.

A subject should be considered for discontinuation, if:

1. The subject is lost to follow-up despite reasonable efforts by the investigator to locate the subject,
2. The subject withdraws consent for further treatment,
3. A liver abnormality occurs [see Appendix 12.3],
4. A renal abnormality occurs [Appendix 12.4],
5. The subject is noncompliant with the protocol based on the investigator or medical monitor assessment,
6. The subject experiences an AE, clinical laboratory abnormality, intercurrent illness or significant worsening of intercurrent illness which, in the opinion of the Investigator, indicates that continued participation in the clinical study is not in the best interest of the subject.

The Sponsor or delegated CRO should be consulted for all cases mentioned above.

A clear and concise reason for discontinuation should be recorded in the eCRF.

6.2 Discontinuation of the Site

If an investigator intends to discontinue participation in the study, the investigator must immediately inform the Sponsor.

6.3 Discontinuation of the Study

The Sponsor may terminate this study prematurely, either in its entirety or at any study site, for reasonable cause provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns. If the Sponsor terminates the study for safety reasons, the Sponsor will immediately notify the investigator and subsequently provide written instructions for study termination.

7 STATISTICAL METHODOLOGY

The statistical analysis will be coordinated by the responsible biostatistician of APGD-US. A statistical analysis plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before the database soft lock at the latest. Any changes from the analyses planned in SAP will be justified in the clinical study report (CSR).

Prior to Database Lock, a Final Review of Data and TLFs Meeting will be held to allow a review of the clinical trial data and to verify the data that will be used for analysis set classification. If required, consequences for the statistical analysis will be discussed and

documented. A meeting to determine analysis set classifications may also be held prior to database lock.

In general, all data will be summarized with descriptive statistics (number of subjects, mean, standard deviation, minimum, median, and maximum) for continuous endpoints, and frequency and percentage for categorical endpoints.

7.1 Sample Size

A sample size of 40 subjects will provide 84% power to detect a difference of 60 seconds in CWR duration from period baseline for CK-2127107 versus placebo with a 1-sided alpha error of 0.05, assuming the standard deviation for change from period baseline is 100 seconds. In order to maintain the statistical power of 80%, approximately 36 subjects would need to complete period 1. Should the discontinuation rate in the first treatment period exceed 10% (approximately 5 subjects), additional subjects will need to be recruited to account for the discontinued subjects.

7.2 Analysis Set

Detailed criteria for analysis sets will be laid out in Classification Specifications and the allocation of subjects to analysis sets will be determined prior to database hard-lock except Pharmacokinetic Analysis Set (PKAS). The allocation of subjects to PKAS will be determined after database hard lock.

7.2.1 Full Analysis Set

The full analysis set (FAS) will consist of all subjects who are randomized and receive at least 1 dose of study drug and have at least 1 baseline measurement and 1 post baseline measurement within a period. Subjects will be included in the treatment arm based on actual treatment received. This will be the primary analysis set for efficacy analyses.

7.2.2 Safety Analysis Set

For the statistical summary of the safety data, the safety analysis set (SAF) will be used. The SAF consists of all subjects who took at least 1 dose of study drug. Subjects will be included in the treatment arm based on actual treatment received.

7.2.3 Pharmacokinetic Analysis Set

The pharmacokinetic analysis set (PKAS) consists of the administered population for which sufficient plasma concentration data is available to facilitate derivation of at least 1 pharmacokinetic parameter and for whom the time of dosing on the day of sampling is known.

7.3 Demographics and Other Baseline Characteristics

Demographics and other baseline characteristics will be summarized by treatment sequence group for the FAS, SAF and PKAS. Descriptive statistics will include number of subjects, mean, standard deviation, minimum, Q1, median, Q3 and maximum for continuous endpoints, and frequency and percentage for categorical endpoints.

7.4 Analysis of Efficacy

Efficacy analysis will be conducted on the FAS. The interpretation of results from statistical tests will be based on the FAS.

7.4.1 Analysis of Primary Endpoint

7.4.1.1 Primary Analysis

The primary endpoint is the difference in change from period baseline in CWR endurance time at the end of each period. The primary analysis for the change from period baseline in CWR endurance time will be a mixed effect model with subject as a random effect and treatment and period as fixed effects. A mixed effect model is a standard model for a crossover study. It improves efficiency by taking in to account both within-subject and between-subject variances. Two-sided 90% confidence interval will be provided based on this model. In addition, statistical tests will be performed based on the model. The hypothesis for comparisons is given as follows:

H0: The change from baseline at the end of period for CK-2127107 and placebo are the same

H1: The change from baseline at the end of period for CK-2127107 and placebo are not the same

Comparisons will be performed at a 2-sided 0.10 significance level. The primary analysis will use the FAS.

7.4.1.2 Sensitivity Analysis

The same analysis of the primary endpoint as described in [Section 7.4.1.1] will be conducted with a model that adds carry-over as a fixed effect to the primary analysis model.

7.4.1.3 Subgroup Analysis

The analysis of the primary variable will be repeated separately by gender and age group. Other subgroup analyses might be added to the SAP.

7.4.2 Analysis of Secondary Endpoints

Analysis of secondary endpoints will be performed based on similar model with the primary endpoint. The analysis set will be the FAS.

7.4.3 Analysis of Exploratory Endpoints

Analysis will be performed based on similar model with the primary endpoint. The analysis set will be the FAS.

7.5 Analysis of Safety

Safety analysis will be performed using the SAF.

7.5.1 Adverse Events

AEs will be coded using MedDRA. The number and percentage of AEs, SAEs, AEs leading to discontinuation, and AEs related to study drug will be summarized by system organ class, preferred term and treatment. The number and percentage of AEs by severity will also be summarized. All AEs will be listed.

7.5.2 Laboratory Assessments

For quantitative laboratory measurements descriptive statistics will be used to summarize results and change from period baseline by treatment and time point. Shifts relative to normal ranges from baseline to each time point during treatment period in laboratory tests will also be tabulated. Laboratory data will be displayed in listings.

7.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from period baseline by treatment and time point. Vital signs data will be displayed in listings.

7.5.4 ECGs

The number and percentage of subjects with normal and abnormal results as assessed by 12-lead central ECG will be tabulated by treatment and time point. Number and percentage of subjects with 12-lead ECG abnormalities will be summarized by treatment group and time point. The 12-lead ECG results will be displayed in data listings.

7.6 Analysis of Pharmacokinetics

Descriptive statistics (e.g., number of subjects, mean, standard deviation, minimum, median, maximum, coefficient of variation and geometric mean) will be provided for pharmacokinetic parameter (day 14 C_{trough} and C_{6h} and day 1 [only for treatment period 2] predose plasma concentration) of CK-2127107 and possible metabolite(s) (if applicable).

7.7 Protocol Deviations and Other Analyses

7.7.1 Protocol Deviations

Protocol deviations as defined in [Section 8.1.6] will be summarized for all randomized subjects by treatment sequence and total as well as by site. A data listing will be provided by site and subjects.

The protocol deviation criteria will be uniquely identified in the summary table and listing. The unique identifiers will be as follows:

PD1 - Entered into the study even though they did not satisfy entry criteria,

PD2 - Developed withdrawal criteria during the study and was not withdrawn,

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment,

PD5 – Plasma samples were not drawn.

7.7.2 Other Analyses

Exploratory biomarker analysis evaluating the correlation between proteomic and/or metabolomic profiles and CK-2127107 drug response will be specified in a separate analysis plan. Any results will be summarized in a report separate from the CSR.

7.8 Interim Analysis (and Early Discontinuation of the Clinical Study)

No formal interim analysis is planned.

7.9 Handling of Missing Data, Outliers, Visit Windows, and Other Information

Missing efficacy data will not be imputed.

See the SAP for details of the definitions for visit time windows to be used for analyses by visit.

8 OPERATIONAL AND ADMINISTRATIVE CONSIDERATIONS

8.1 Procedure for Clinical Study Quality Control

8.1.1 Data Collection

The investigator or site designee will enter data collected using an electronic data capture (EDC) system. In the interest of collecting data in the most efficient manner, the investigator or site designee should record data (including laboratory values, if applicable) in the eCRF within 10 days after the subject visit.

The investigator or site designee is responsible to ensure that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with source documents. These documents should be appropriately maintained by the site.

The monitor should verify the data in the eCRFs with source documents and confirm that there are no inconsistencies between them.

Laboratory tests are performed at a central laboratory. Laboratory data will be transferred electronically to the Sponsor or designee at predefined intervals during the study. The laboratory will provide the Sponsor or designee with a complete and clean copy of the data.

ECG results are performed at a central ECG reading facility. Central ECG read data will be transferred electronically to the Sponsor or designee at predefined intervals during the study. The central ECG laboratory will provide the Sponsor or designee with a complete and clean copy of the data.

For screen failures, the demographic data, reason for failing, informed consent, inclusion and exclusion criteria and AEs will be collected in the eCRF.

ePRO:

Subject diaries and questionnaires will be completed by the subject on an electronic device (ePRO). The information completed by the subject on the electronic device will be automatically uploaded into a central website. The investigator or site designee should review the diaries and questionnaire data on the website for confirmation of transmittal while the subject is at the site. The diary and questionnaire data will be transferred electronically to the Sponsor or designee at predefined intervals during the study. The vendor, ERT, will provide Sponsor or designee with a complete and clean copy of the data.

8.1.2 Specification of Source Documents

Source data must be available at the site to document the existence of the study subjects and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the subject.

The following information should be included in the source medical records:

- Demographic data (age, sex, race, ethnicity, height and body weight)
- Inclusion and exclusion criteria details
- Participation in study and original signed and dated ICFs
- Visit dates
- Medical history and physical examination details
- Key efficacy and safety data (as specified in the protocol)
- AEs and concomitant medication
- Results of relevant examinations (e.g., ECG charts, X-ray films etc.)
- Laboratory printouts (if applicable)
- Test assessment printouts: CWR, IET, MIP,MEP, SPPB and physical activity
- Site created source document for spirometry
- Dispensing and return of study drug details
- Reason for premature discontinuation (if applicable)
- Randomization number (if applicable)

8.1.3 Clinical Study Monitoring

The Sponsor or delegated CRO is responsible for monitoring the clinical study to ensure that subject's human rights, safety, and well-being are protected, that the study is properly conducted in adherence to the current protocol and GCP, and study data reported by the investigator/subinvestigator are accurate and complete and that they are verifiable with study-related records such as source documents. The Sponsor is responsible for assigning study monitor(s) to this study for proper monitoring. They will monitor the study in accordance with planned monitoring procedures.

8.1.4 Direct Access to Source Data/Documents

The investigator and the site must accept monitoring and auditing by the Sponsor or delegated CRO as well as inspections from the IRB/IEC and relevant regulatory authorities. In these

instances, they must provide all study-related records, such as source documents [refer to Section 8.1.2] when they are requested by the Sponsor monitors and auditors, the IRB/IEC, or regulatory authorities. The confidentiality of the subject's identities shall be well protected consistent with local and national regulations when the source documents are subject to direct access.

8.1.5 Data Management

Data Management will be coordinated by the Data Science department of the Sponsor in accordance with the SOPs for data management. All study specific processes and definitions will be documented by Data Management. eCRF completion will be described in the eCRF instructions. Coding of medical terms and medications will be performed using MedDRA and WHO Drug Dictionary respectively.

8.1.6 Protocol Deviations

A protocol deviation is generally an unplanned excursion from the protocol that is not implemented or intended as a systematic change. The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol and must protect the rights, safety and welfare of subjects. The investigator should not implement any deviation from, or changes of, the protocol, unless it is necessary to eliminate an immediate hazard to study subjects.

A protocol waiver is a documented prospective approval of a request from an investigator to deviate from the protocol. Protocol waivers are strictly prohibited.

For the purposes of this protocol, deviations requiring notification to Sponsor are defined as any subject who:

- Entered into the study even though they did not satisfy entry criteria
- Developed withdrawal criteria during the study and not withdrawn
- Received wrong treatment or incorrect dose
- Received excluded concomitant treatment
- Plasma samples were not drawn

When a deviation from the protocol is identified for an individual subject, the investigator or designee must ensure the Sponsor is notified. The Sponsor will follow-up with the investigator, as applicable, to assess the deviation and the possible impact to the safety and / or efficacy of the subject to determine subject continuation in the study.

If a deviation impacts the safety of a subject, the investigator must contact the Sponsor immediately.

The investigator will also assure that deviations meeting IRB/IEC and applicable regulatory authorities' criteria are documented and communicated appropriately. All documentation and communications to the IRB/IEC and applicable regulatory authorities will be provided to the Sponsor and maintained within the trial master file.

NOTE: Other deviations outside of the categories defined above that are required to be reported by the IRB/IEC in accordance with local requirements will be reported, as applicable.

8.1.7 End of Trial in All Participating Countries

The end of study in all participating countries is defined as the last subject's last visit.

8.2 Ethics and Protection of Subject Confidentiality

8.2.1 Institutional Review Board (IRB) / Independent Ethics Committee (IEC) / Competent Authorities (CA)

The clinical study may begin after acquisition of a written approval from IRB/IEC.

GCP requires that the clinical protocol, any protocol amendments, the IB, the ICF and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an IEC/IRB. The IEC/IRB will review the ethical, scientific and medical appropriateness of the study before it is conducted. IEC/IRB approval of the protocol, informed consent and subject information and/or advertising, as relevant, will be obtained prior to the authorization of drug shipment to a study site.

Any substantial amendments to the protocol will require IEC/IRB approval prior to implementation of the changes made to the study design at the site. The investigator will be required to submit, maintain and archive study essential documents according to ICH GCP.

Any SAEs that meet reporting criteria, as dictated by local regulations, will be reported to both responsible Ethics Committees and regulatory agencies, as required. During the conduct of the study, the investigator should promptly provide written reports (e.g., ICH Expedited Reports and any additional reports required by local regulations) to the IEC/IRB of any changes that affect the conduct of the study and/or increase the risk to subjects. Written documentation of the submission to the IEC/IRB should also be provided to Sponsor.

If required by local regulations, the investigator shall make accurate and adequate written progress reports to the IEC/IRB at appropriate intervals, not exceeding 1 year. The investigator shall make an accurate and adequate final report to the IRB/IEC within 90 days after the close-out visit for APGD-sponsored studies after last subject out or termination of the study.

8.2.2 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical principles that have their origin in the Declaration of Helsinki.

8.2.3 Informed Consent of Subjects

8.2.3.1 Subject Information and Consent

The investigator or his/her representative will explain the nature of the study to the subject or his/her guardian or legal representative, and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent

statement will be reviewed and signed and dated by the subject or his/her guardian or legal representative, the person who administered the informed consent and any other signatories according to local requirements. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that informed consent was obtained prior to any study-related procedures and that the subject received a signed copy.

The signed consent forms will be retained by the investigator and made available (for review only) to the study monitor and auditor regulatory authorities and other applicable individuals upon request.

8.2.3.2 Supply of New and Important Information Influencing the Subject's Consent and Revision of the Written Information

1. The investigator or his/her representative will immediately inform the subject orally whenever new information becomes available that may be relevant to the subject's consent or may influence the subject's willingness to continue to participate in the study (e.g., report of serious drug adverse drug reaction). The communication must be documented in the subject's medical records and must document whether the subject is willing to remain in the study or not.
2. The investigator must update their ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent from the subject on all updated ICFs throughout their participation in the study. The investigator or his/her designee must reconsent subjects with the updated ICF even if relevant information was provided orally. The investigator or his/her representative who obtained the written informed consent and the subject should sign and date the ICF. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must be made in the subject's records documenting the re-consent process.

8.2.4 Subject Confidentiality

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Such medical information may be given only after approval of the subject to the subject's physician or to other appropriate medical personnel responsible for the subject's well-being.

The Sponsor shall not disclose any confidential information on subjects obtained during the performance of their duties in the clinical study without justifiable reasons.

The Sponsor affirms the subject's right to protection against invasion of privacy. Only a subject identification number and/or initials will identify subject data retrieved by the Sponsor. However, the Sponsor requires the investigator to permit the Sponsor, Sponsor's representative(s), the IRB/IEC and when necessary, representatives of the regulatory health authorities to review and/or to copy any medical records relevant to the study.

The Sponsor will ensure that the use and disclosure of protected health information obtained during a research study complies with the federal and/or regional legislation related to the privacy and protection of personal information (i.e., HIPAA).

8.3 Administrative Matters

8.3.1 Arrangement for Use of Information and Publication of the Clinical Study

Information concerning the study drug, patent applications, processes, unpublished scientific data, the IB and other pertinent information is confidential and remains the property of the Sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the Sponsor will use the information obtained during the clinical study in connection with the development of the drug and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this clinical study, the investigator understands that he/she has an obligation to provide the Sponsor with all data obtained during the study.

Publication of the study results is discussed in the clinical study agreement.

8.3.2 Documents and Records Related to the Clinical Study

The investigator will archive all study data (e.g., subject identification code list, source data, electronic data sources, and Investigator's File) and relevant correspondence. These documents are to be kept on file for the appropriate term determined by local regulation (for US sites, 2 years after approval of the NDA or discontinuation of the IND). The Sponsor will notify the site/investigator if the NDA/MAA/J-NDA is approved or if the IND/IMPD/CHIKEN TODOKE is discontinued. The investigator agrees to obtain the Sponsor's agreement prior to disposal, moving, or transferring of any study-related records. The Sponsor will archive and retain all documents pertaining to the study according to local regulations.

Data generated by the methods described in the protocol will be recorded in the subjects' medical records and/or study progress notes. Data will be entered on the eCRFs supplied for each subject.

The investigator and Sponsor will mutually agree upon the storage format for the retention of electronic data.

8.3.3 Protocol Amendment and/or Revision

Any changes to the study that arise after approval of the protocol must be documented as protocol amendments: substantial amendments and/or non-substantial amendments. Depending on the nature of the amendment, either IRB/IEC, Competent Authority approval or notification may be required. The changes will become effective only after the approval of the Sponsor, the investigator, the regulatory authority, and the IRB/IEC (if applicable).

Amendments to this protocol must be signed by the Sponsor and the investigator. Written verification of IRB/IEC approval will be obtained before any amendment is implemented

which affects subject safety or the evaluation of safety, and/or efficacy or pharmacokinetics. Modifications to the protocol that are administrative in nature do not require IRB/IEC approval, but will be submitted to the IRB/IEC for their information, if required by local regulations.

If there are changes to the informed consent, written verification of IRB/IEC approval must be forwarded to the Sponsor. An approved copy of the new Informed Consent must also be forwarded to the Sponsor.

8.3.4 Insurance of Subjects and Others

The Sponsor has covered this study by means of an insurance of the study according to national requirements. The name and address of the relevant insurance company, the certificate of insurance, the policy number and the sum insured are provided in the Investigator's File.

8.3.5 Signatory Investigator for Clinical Study Report

ICH E3 guidelines recommend and EU Directive 2001/83/EC requires that a final study report which forms part of a marketing authorization application be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator (s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study. The representative for coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the Sponsor prior to database lock.

9 QUALITY ASSURANCE

The Sponsor is implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP and applicable regulatory requirement(s).

The Sponsor or Sponsor's designee may arrange to audit the clinical study at any or all investigational sites and facilities. The audit may include on-site review of regulatory documents, eCRFs and source documents. Direct access to these documents will be required by the auditors.

10 STUDY ORGANIZATION

10.1 Independent Data-Monitoring Committee (IDMC) | Data and Safety Monitoring Board (DSMB) | Monitoring Committee | Other Evaluation Committee(s)

Not applicable.

10.2 Other Study Organization

Not applicable.

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12 APPENDICES

12.1 List of Excluded Concomitant Medications/Foods

CYP3A4 Strong Inhibitors	**CYP3A4 Inducers**	**OCT1 Substrates**	**OCT2 Substrates**
Indinavir	Carbamazepine	Oxaliplatin	Pindolol
Nelfinavir	Efavirenz	Dofetilide	Varenicline
Ritonavir	Nevirapine		Pilsicainide
Clarithromycin	Phenobarbital		
Itraconazole	Phenytoin		
Ketoconazole	Pioglitazone		
Nefazodone	Rifabutin		
Grapefruit juice	Rifampin		
Seville oranges	St. John's Wort		
	Troglitazone		

12.2 List of Concomitant Medication to be Used With Caution

OCT1 Substrates	**OCT1/2 Substrates**
Aciclovir	Metformin
Ganciclovir	

12.3 Liver Safety Monitoring and Assessment

Any subject enrolled in a clinical study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 × ULN, or bilirubin > 2 × ULN, should undergo detailed testing for liver enzymes (including at least ALT, AST, ALP, and TBL). Testing should be repeated within 48 to 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the investigator, study monitor and study team. Subjects should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN:

	ALT or AST		**Total Bilirubin**
Moderate	> 3 × ULN	or	> 2 × ULN
Severe*	> 3 × ULN	and	> 2 × ULN

In addition, the subject should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and International Normalized Ratio (INR) > 1.5 (if INR testing is applicable/evaluated).
- ALT or AST > 3× ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

The investigator may determine that abnormal LFTs, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and laboratory tests. The site should complete the liver abnormality case report form (LA-CRF) that has been developed globally and can be activated for any study or an appropriate document. Subjects with confirmed abnormal LFTs should be followed as described below.

Confirmed moderately abnormal LFTs should be repeated 2 to 3 times weekly then weekly or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as an SAE.

The Sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to study drug are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new onset-diseases is to be recorded as 'adverse events' on the AE page of the eCRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic, and/or diabetic subjects and may be associated with fluctuating aminotransferase levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications, including dose, are to be entered on the concomitant medication page of the eCRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the subject's history, other testing may be appropriate including:
    - Acute viral hepatitis (A,B, C, D, E or other infectious agents)
    - Ultrasound or other imaging to assess biliary tract disease
    - Other laboratory tests including INR, direct bilirubin
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Study Discontinuation

In the absence of an explanation for increased LFTs, such as viral hepatitis, pre-existing or acute liver disease or exposure to other agents associated with liver injury, the subject may be discontinued from the study. The investigator may determine that it is not in the subject's best interest to continue study enrollment. Discontinuation of treatment should be considered if:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and TBL > 2 × ULN or INR > 1.5) (if INR testing is applicable/evaluated)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

In addition, if close monitoring for a subject with moderate or severe hepatic laboratory tests is not possible, study drug should be discontinued.

*Hy's Law Definition: Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10 to 50% mortality

(or transplant). The 2 "requirements" for Hy's Law are: 1) Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in transaminase elevations higher than 3 × the ULN ("2 × ULN elevations are too common in treated and untreated subjects to be discriminating"). 2) Cases of increased bilirubin (at least 2 x ULN) with concurrent transaminase elevations at least 3 × ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated ALP) or Gilbert's syndrome [Temple, 2006].

References

Guidance for Industry titled "Drug-Induced Liver Injury: Premarketing Clinical Evaluation" issued by FDA on July 2009.

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12.4 Renal Safety Monitoring

As noted in previous clinical studies with CK-2127107 have laboratory values for creatinine increased in a dose and concentration dependent fashion. The elevated values appear to slowly return toward normal and no associated symptoms or AEs were reported. It is possible that CK-2127107 or 1 of its metabolites may interfere with either the creatinine assay [Ducharme et al, 1993] or may inhibit the renal tubular secretion of creatinine, as has been reported with other drugs including cimetidine [Agarwal, 1993; Hilbrands et al, 1993; Payne, 1993; van Acker et al, 1993; Schutzer et al, 2010]. The inhibition of renal tubular OCT2 by CK-2127107 could also be a potential factor of increases in serum creatinine.

Subjects shall be discontinued from the study drug should their serum creatinine demonstrate an increase of 1.5 to 2.0 times above baseline (CTCAE grade 1) due to study drug[a] in concordance with an increase in serum cystatin C levels.

References:

Agarwal R. Creatinine clearance with cimetidine for measurement of GFR. Lancet. 1993;341(8838):188.

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van Acker BA, Koomen GC, Koopman MG, de Waart DR, Arisz L. Creatinine clearance with cimetidine for measurement of GFR. Lancet. 1993;341(8852):1089-90.

[a] Patients should be evaluated first for potential other reasons of elevated serum creatinine and cystatin C levels (i.e., dehydration, concomitant medication or any underlying pathology that could explain the increase in renal markers).

12.5 Common Serious Adverse Events

For this protocol, there is no list of common SAEs anticipated for the study population for the purposes of IND safety reporting.

12.6 Clinical Laboratory Tests

Visit	Type	Analyte
Screening, Day 1, Day 6[a], Day 14, EOT/ED, Follow-up	Biochemistry	Sodium Potassium Calcium Chloride Glucose Creatinine ALP AST ALT GGT TBL Total protein Albumin BUN Cholesterol LDH Magnesium Phosphorus Total CPK Triglycerides Uric acid Cystatin C
Screening, Day 1, Day 14, EOT/ED, Follow-up	Urinalysis	Protein Glucose pH Blood Bilirubin Ketones Leukocytes Nitrite Urobilinogen Specific Gravity
Screening, Day 1, Day 14, EOT/ED, Follow-up	Hematology	Hematocrit Hemoglobin MCH MCV MPV Platelet count Red blood cell distribution width Red blood cell count WBC count WBC differential

Table continued on next page

Visit	Type	Analyte
Screening, Day 1	Drug screening (Urine)	Amphetamines Barbiturates Benzodiazepines Cocaine Marijuana/Cannabinoids (THC) Methadone Opiates Phencyclidine
Screening, Day1	Alcohol screening	Alcohol
Screening, EOT/ED, Follow-up	Pregnancy test	β-HCG
Screening	Serology	HBsAg HCV antibody HIV antibody

β-HCG: β-human chorionic gonadotropin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CPK: creatine phosphokinase; EOT: end of treatment; ED: early discontinuation; GGT: γ-glutamyl transferase; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus; HIV: human immunodeficiency virus; LDH: lactate dehydrogenase; MCH: mean corpuscular hemoglobin; MCV: mean corpuscular volume; MPV: mean platelet volume; TBL: total bilirubin; THC: tetrahydrocannabinol; WBC: white blood cell

a. Serum samples for monitoring creatinine, BUN, cystatin C and liver enzymes (renal and liver safety assessments) will be collected on day 6.

12.7 Retrospective PGx Substudy

INTRODUCTION

PGx research aims to provide information regarding how naturally occurring changes in a subject's gene and/or expression based on genetic variation may impact what treatment options are best suited for the subject. Through investigation of PGx by technologies such as genotyping, gene sequencing, statistical genetics and Genome-Wide Association Studies, the relationship between gene profiles and a drug's kinetics, efficacy or toxicity may be better understood. As many diseases may be influenced by 1 or more genetic variations, PGx research may identify which genes are involved in determining the way a subject may or may not respond to a drug.

OBJECTIVES

The PGx research that may be conducted in the future with acquired blood samples is exploratory. The objective of this research will be to analyze or determine genes of relevance to clinical response, pharmacokinetics and toxicity/safety issues.

By analyzing genetic variations, it may be possible to predict an individual subject's response to treatment in terms of efficacy and/or toxicity.

SUBJECT PARTICIPATION

Subjects who have consented to participate in this study may participate in this PGx substudy. As part of this substudy, subjects must provide written consent prior to providing any blood samples that may be used at a later time for genetic analysis.

SAMPLE COLLECTION AND STORAGE

Subjects who consent to participate in this substudy will provide one 4-mL tube of whole blood per Astellas' instructions. Each sample will be identified by the unique subject number (first code). Samples will be shipped frozen to a designated banking CRO either directly from site or via a central laboratory as directed by Astellas.

PGx ANALYSIS

Details on the potential PGx analysis cannot be established yet. Astellas may initiate the PGx analysis in case evidence suggests that genetic variants may be influencing the drug's kinetics, efficacy and/or safety.

DISPOSAL OF PGx SAMPLES / DATA

All PGx samples collected will be stored for a period of up to 15 years following study database hardlock. If there is no requirement for analysis, the whole blood sample will be destroyed after the planned storage period. The subject has the right to withdraw consent at any time. When a subject's withdraw notification is received, the PGx sample will be destroyed. The results of any PGx analysis conducted on a sample prior to its withdrawal will be retained at Astellas indefinitely.

INFORMATION DISCLOSURE TO THE SUBJECTS

Exploratory PGx analysis may be conducted following the conclusion of the clinical study, if applicable. The results of the genetic analysis will not be provided to any investigators or subjects, nor can the results be requested at a later date. Any information that is obtained from the PGx analysis will be the property of Astellas.

12.8 mMRC Scale

The mMRC scale is a modification of the scale originally defined in 1959 by Fletcher et al as the first clinical scale for the determination of dyspnea. It is a 5-point scale based on the sensation of breath difficulty experienced by the subject during daily life activities:

Grade	Description
0	I only get breathless with strenuous exercise.
1	I get short of breath when hurrying on the level or walking up a slight hill.
2	I walk slower than people of the same age on the level because of breathlessness, or I have to stop for breath when walking at my own pace on the level.
3	I stop for breath after walking about 100 meters or after a few minutes on the level.
4	I am too breathless to leave the house or I am breathless when dressing or undressing.

References

Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (Updated 2015) [Internet]. [cited 2015 Oct 22]. Available from: http://www.goldcopd.org/.

12.9 Borg CR10

Rating of Perceived Exertion	Description
0	Complete rest
1	Very, very easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	
7	Very Hard
8	
9	
10	Extremely Hard (almost maximal)
---	Exhaustion

---: maximal

References

Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14(5):377-81.

13 ATTACHMENT 1: NON-SUBSTANTIAL AMENDMENT 1

I. The purpose of this amendment is:

1. Remove Accelerometer Test and Leg Extension Strength Test
DESCRIPTION OF CHANGE:
Remove accelerometer test and leg extensor strength test from the exploratory objectives and endpoints.
RATIONALE:
To streamline assessments, exploratory endpoints that would not significantly contribute to the validity of the primary endpoint are removed.
2. Increase Number of Study Sites
DESCRIPTION OF CHANGE:
Revise the number of study sites to “approximately 4”.
RATIONALE:
The number of sites is amended to allow approximately 2 additional sites to participate in the study.
3. Clarify Number of Subjects Randomized in the Study
DESCRIPTION OF CHANGE:
Specify that approximately 40 subjects “are planned to be randomized,” rather than “will be randomized.”
RATIONALE:
This change is made to allow for recruitment of additional subjects if the discontinuation rate in the first treatment period exceeds 5 subjects, in order to maintain the statistical power of 80%.
4. Specify Assessment of Physical Activity
DESCRIPTION OF CHANGE:
Add language to specify that physical activity will be assessed using an online 12-item patient reported outcome (PRO) questionnaire (7-day recall clinical visit version of PRO active physical activity in COPD [C-PPAC]) due to the removal of the accelerometer.
RATIONALE:
The accelerometer test is removed from the protocol as specified in non-substantial change No. 1, therefore additional detail regarding continued use of the online 12-item PRO questionnaire is added for clarification.

5. Update Physical Examination
DESCRIPTION OF CHANGE:
Revise Section 5.4.4. (Physical Examination) to state that a full physical examination will be performed at screening and visit 10, while a symptom-directed physical examination will be performed at the remaining visits 4, 6, 7, and 9.
RATIONALE:
This wording is updated to clarify at which visits the full physical examination is required and which visits will allow a symptom-directed exam, based on the discretion of the study physician. Language is added to specify that a study physician (MD or DO) will be able to delegate this task to a nurse practitioner and/or physician assistant per institutional policy.
6. Revise Definition of Full Analysis Set
DESCRIPTION OF CHANGE:
Revise the definition of Full Analysis Set (FAS) to state that subjects will be included in this treatment arm based on actual treatment received.
RATIONALE:
The purpose of the study is to understand the action of the study drug; therefore, it is more meaningful to include subjects in the FAS based on treatment received.
7. Minor Administrative-type Changes
DESCRIPTION OF CHANGE:
Include minor administrative-type changes (e.g., typos, format, numbering and consistency throughout the protocol) and update the List of Abbreviations, Flow Chart, and Schedule of Assessments.
RATIONALE:
These changes are made to provide clarifications to the protocol and to ensure complete understanding of study procedures.

II. Amendment Summary of Changes:

IV Synopsis, Study Objective(s) and 2 Study Objective(s), Design and Endpoints *2.1 Study Objectives*
WAS:
Secondary Objectives ● To assess cardiopulmonary and neuromuscular effects of CK-2127107 relative to placebo on: o The change from period baseline in oxygen uptake (VO_2), ventilation (V_E), ventilatory equivalent for carbon dioxide (V_E/VCO_2) and other breath-by-breath cardiometabolic variables, inspiratory capacity (IC) and perceived exertion for dyspnea and leg discomfort (Borg CR10), at isotime and peak during CWR test o Activation of accessory respiratory muscles (by electromyogram [EMG]) at isotime* and peak exercise during CWR test ● To assess the effect of CK-2127107 on resting spirometry relative to placebo ● To assess the safety and tolerability of CK-2127107 ● To assess the pharmacokinetics of CK-2127107 Exploratory Objectives ● To explore the effects of CK-2127107 relative to placebo on: o Patient reported outcomes (PROs) and physical activity assessed by an accelerometer o Physical performance via a short physical performance battery (SPPB) and leg muscle strength via a leg extensor strength test (1 repetition maximum [1RM]) o Respiratory muscle strength by maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) *Isotime is the shortest duration of all of the CWR tests performed before and after treatment, not including screening.
IS AMENDED TO:
Secondary Objectives ● To assess cardiopulmonary and neuromuscular effects of CK-2127107 relative to placebo on: o The change from period baseline in oxygen uptake (VO_2), ventilation (V_E), ventilatory equivalent for carbon dioxide (V_E/VCO_2) and other breath-by-breath cardiometabolic variables, inspiratory capacity (IC) and perceived exertion for dyspnea and leg discomfort (Borg CR10), at isotime* and peak during CWR test o Activation of accessory respiratory muscles (by electromyogram [EMG]) at isotime* and peak exercise during CWR test ● To assess the effect of CK-2127107 on resting spirometry relative to placebo ● To assess the safety and tolerability of CK-2127107 ● To assess the pharmacokinetics of CK-2127107 ***Isotime is the shortest duration of all of the CWR tests performed before and after**

treatment, not including screening.

Exploratory Objectives

- To explore the effects of CK-2127107 relative to placebo on:
  - Patient reported outcomes (PROs) ~~and physical activity assessed by an accelerometer~~
  - Physical performance via a short physical performance battery (SPPB) ~~and leg muscle strength via a leg extensor strength test (1 repetition maximum [1RM])~~
  - Respiratory muscle strength by maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP)

  ~~*Isotime is the shortest duration of all of the CWR tests performed before and after treatment, not including screening.~~

IV Synopsis, Number of Subjects to be Enrolled/Randomized
WAS:
Approximately 40 subjects will be randomized.
IS AMENDED TO:
Approximately 40 subjects **are planned to** ~~will~~ be randomized.

IV Synopsis, Planned Total Number of Study Centers and Location(s) and 3 Study Population *3.1 Selection of Study Population*
WAS:
Approximately 40 subjects will be randomized to 1 of 2 treatment sequences in a crossover fashion. Enrollment for this study will be at 1 to 2 sites in the United States and possibly Canada or the United Kingdom.
IS AMENDED TO:
Approximately 40 subjects **are planned to** ~~will~~ be randomized to 1 of 2 treatment sequences in a crossover fashion. Enrollment for this study will be at ~~1 to 2~~ **approximately 4** sites in the United States and possibly Canada or the United Kingdom.

IV Synopsis, Study Design Overview and 2 Study Objective(s), Design and Endpoints *2.2.1 Study Design*
WAS:
After the CWR test, subjects receive an accelerometer, physical activity will be monitored at home for 1 week using an accelerometer combined with a PRO during the screening period (between visit 2 and day -1). The CWR test will be completed at visit 2 at least 24 hours after the IET. The CWR exercise test will be performed at 80% peak WR (rounded to the nearest 5 watts). This screening test is used to confirm the correct WR selection for the rest of the study and to familiarize the

subject with the testing procedure. Subjects are expected to tolerate this WR for durations in the range of 4 to 8 minutes. Establishing a WR that will achieve this specific duration is critical if constant work rate testing is deemed to detect treatment effects in interventional studies [Casaburi R, 2005; Puente-Maestu et al, 2016]. In the event that the tolerable duration is outside this range, a second CWR is performed within the next 5 days at ± 5 watts to determine whether this adjusted WR will result in an endurance time in the desired range. If this cannot be achieved with 2 adjustments (a total of 3 CWR tests), the subject will be classified as a screen failure and no additional assessments completed. Once the WR is established that leads to intolerance in 4 to 8 minutes, all subsequent tests use the WR that led to intolerance. No additional WR adjustments can be made once the WR intolerance level is set.

At visit 3 subjects will return the accelerometer to the clinical unit and a weekly PRO assessment will be completed at the end of the 7-day activity monitoring using a 7-day recall online 12-item questionnaire. If the subject is not compliant, or for some unforeseen reason the accelerometer does not record correctly, the physical activity monitoring may be repeated in the screening period. Visit 3 will be considered optional if the subject successfully completes the CWR at visit 2. Those subjects could also return the accelerometer and complete the weekly PRO assessment (C-PPAC) at visit 4.

On day 1 (visit 4) at baseline, all subjects will complete postbronchodilator spirometry, respiratory muscle strength tests (MIP and MEP), a resting ECG, CWR test (including breath-by-breath cardiometabolic measurements and respiratory muscle EMG), quality of life (QoL) assessments (St. George's respiratory questionnaire for COPD patients [SGRQ-C] and short form-36 [SF-36]), a SPPB and a leg extensor strength test (1RM) to establish period baseline measurements, and will be randomly assigned to 1 of 2 treatment sequences in a 1:1 ratio.

To achieve steady-state exposure to CK-2127107, subjects will receive CK-2127107 (500 mg) or matching placebo twice daily for 14 days, except on day 1 and day 14. On day 1, subjects will take active or placebo drug once in the evening, and on day 14, subjects will take active or placebo drug once in the morning. Subjects will receive an accelerometer at visit 5, and physical activity will be monitored using the accelerometer and a combined PRO during the second week (days 7 to 13). At visit 5, subjects will also have renal and liver safety assessments.

IS AMENDED TO:

~~After the CWR test, subjects receive an accelerometer, physical activity will be monitored at home for 1 week using an accelerometer combined with a PRO during the screening period (between visit 2 and day -1).~~

The CWR test will be completed at visit 2 at least 24 hours after the IET. The CWR exercise test will be performed at 80% peak WR (rounded to the nearest 5 watts). This screening test is used to confirm the correct WR selection for the rest of the study and to familiarize the subject with the testing procedure. Subjects are expected to tolerate this WR for durations in the range of 4 to 8 minutes. Establishing a WR that will achieve this specific duration is critical if constant work rate testing is deemed to detect treatment effects in interventional studies [Casaburi R, 2005; Puente-Maestu et al, 2016]. In the event that the tolerable duration is outside this range, a second CWR is performed within the next 5 days at ± 5 watts

to determine whether this adjusted WR will result in an endurance time in the desired range. If this cannot be achieved with 2 adjustments (a total of 3 CWR tests), the subject will be classified as a screen failure and no additional assessments completed. Once the WR is established that leads to intolerance in 4 to 8 minutes, all subsequent tests use the WR that led to intolerance. No additional WR adjustments can be made once the WR intolerance level is set.

~~At visit 3 subjects will return the accelerometer to the clinical unit and a weekly PRO assessment will be completed at the end of the 7-day activity monitoring using a 7-day recall online 12-item questionnaire. If the subject is not compliant, or for some unforeseen reason the accelerometer does not record correctly, the physical activity monitoring may be repeated in the screening period. Visit 3 will be considered optional if the subject successfully completes the CWR at visit 2. Those subjects could also return the accelerometer and complete the weekly PRO assessment (C-PPAC) at visit 4.~~

On day 1 (visit 4) at baseline, all subjects will complete postbronchodilator spirometry, respiratory muscle strength tests (MIP and MEP), a resting ECG, CWR test (including breath-by-breath cardiometabolic measurements and respiratory muscle EMG), quality of life (QoL) assessments (St. George's respiratory questionnaire for COPD patients [SGRQ-C] and short form-36 [SF-36]), **physical activity assessments (7-day recall 12-item questionnaire), and** a SPPB ~~and a leg extensor strength test (1RM)~~ to establish period baseline measurements, and will be randomly assigned to 1 of 2 treatment sequences in a 1:1 ratio.

To achieve steady-state exposure to CK-2127107, subjects will receive CK-2127107 (500 mg) or matching placebo twice daily for 14 days, except on day 1 and day 14. On day 1, subjects will take active or placebo drug once in the evening, and on day 14, subjects will take active or placebo drug once in the morning. ~~Subjects will receive an accelerometer at visit 5, and physical activity will be monitored using the accelerometer and a combined PRO during the second week (days 7 to 13).~~ At visit 5, subjects will also have renal and liver safety assessments.

IV Synopsis, Inclusion/Exclusion Criteria and 3 Study Population

3.2 Inclusion Criteria

WAS:

5. Subject is able to complete technically acceptable respiratory muscle strength tests, spirometry, muscle strength test, physical performance test and exercise tests.
6. Subject is willing to wear an accelerometer during waking hours in assigned periods during the study.

IS AMENDED TO:

5. Subject is able to complete technically acceptable respiratory muscle strength tests, spirometry, ~~muscle strength test,~~ physical performance test and exercise tests.
6. ~~Subject is willing to wear an accelerometer during waking hours in assigned periods during the study~~. **This criterion has been deleted.**

IV Synopsis, Endpoints for Evaluation and 2 Study Objective(s), Design and Endpoints
2.3.3 Exploratory Endpoints

WAS:

The exploratory endpoints are as follows:

- Change from period baseline in QoL outcomes (SGRQ-C, SF-36) relative to placebo
- Change from baseline in physical activity using the PROactive approach (DynaPort®© accelerometer and combined PRO). This will be assessed during only the first treatment period. Therefore, this will represent the response to placebo for half the subjects and the response to the study drug for the other half of subjects.
- Change from period baseline in physical performance tests battery score (SPPB) relative to placebo
- Change from period baseline in leg muscle strength relative to placebo (as assessed by the maximal weight a subject can lift with 1 repetition [1RM])
- Change from period baseline in MIP and MEP relative to placebo. This test is performed before exercise testing and is part of the resting assessment.

IS AMENDED TO:

The exploratory endpoints are as follows:

- Change from period baseline in QoL outcomes (SGRQ-C, SF-36) relative to placebo
- Change from baseline in physical activity using **a 7-day recall online 12-item questionnaire.** ~~the PROactive approach (DynaPort®© accelerometer and combined PRO).~~ This will be assessed during only the first treatment period. Therefore, this will represent the response to placebo for half the subjects and the response to the study drug for the other half of subjects.
- Change from period baseline in physical performance tests battery score (SPPB) relative to placebo
- ~~Change from period baseline in leg muscle strength relative to placebo (as assessed by the maximal weight a subject can lift with 1 repetition [1RM])~~
- Change from period baseline in MIP and MEP relative to placebo. This test is performed before exercise testing and is part of the resting assessment.

V Flow Chart and Schedule of Assessments
Table 1 Schedule of Assessments

WAS:

Assessments	Screening				Treatment Period 1				Washout	Treatment Period 2			Follow-up
			Baseline for physical activity										
Day	-28 to -9	-27 to -8	-26 to -1[a]	-19 to -1	1	6	7 to 13	14	14 days	1	6	EOT[b]/ ED	7 days after EOT/ED
Window	-	-	-	-	-	+3	-	+3	+7	-	+3	+3	+7
Visit Number	1	2	-	3[c]	4	5	-	6	-	7	8	9	10
Informed consent	X												
Inclusion/Exclusion	X												
Medical history	X				X					X			

Demographics	X												
Drug and alcohol screen[d]	X				X					X			
Randomization					X								
Dispense/Collect study drug					X			X		X		X	
Administration of study drug[e]					←				→	←			→
Physical examination	X				X			X		X		X	X
Vital signs[f]	X				X			X		X		X	X
Hematology	X				X			X		X		X	X
Serum chemistries	X				X	X[g]		X		X	X[g]	X	X
Serology	X												
Urinalysis	X				X			X		X		X	X
Serum pregnancy test	X											X	X
Spirometry[h]	X				X			X		X		X	X
Respiratory muscle strength tests (MIP, MEP)					X			X		X		X	
12-lead ECG	X				X			X		X		X	X
CWR exercise test		X[i]			X			X		X		X	
QoL outcomes (SGRQ-C, SF-36)					X			X		X		X	
Physical performance test (SPPB)					X			X		X		X	
Leg extensor strength test (1RM)					X			X		X		X	
IET	X												
Supply acclerometer		X[j]				X							
Physical activity monitoring (Accelerometer and PRO)[k]			←		→		←	→					
Return accelerometer and complete PRO				X	X			X					
Blood sampling for pharmacokinetics[l]								Predose6h		Predose		Predose 6h	
Optional blood sampling for pharmacogenomics[m]					X								
Concomitant medication	X	X		X	X	X	X	X	X	X		X	X
Adverse events assessment	X	X		X	X	X	X	X	X	X		X	X

CWR: constant work rate; ECG: electrocardiogram; ED: early discontinuation; EOT: end of treatment; IET: incremental exercise test; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; PRO: patient reported outcome; 1RM: 1 repetition maximum; SF-36: Short Form-36; SGRQ-C: St. George's Respiratory Questionnaire for COPD; SPPB: short physical performance battery

a. Physical activity will be monitored at home for 7 days (1 week) and may occur between days -26 to -1. If the subject was not compliant or for some unforeseen reason the accelerometer did not record correctly, the physical activity monitoring and the assessment of a weekly PRO (7-day recall) may be repeated in the screening period.

b. EOT: day 14 of treatment period 2

c. Visit 3 will be considered optional if the subject successfully completes the CWR at visit 2. Subjects who do not attend visit 3 will resume their visit schedule with visit 4.

d. A standard urine screen for drugs of abuse (e.g., cannabinoids, cocaine and amphetamines) and benzodiazepines, and blood, urine, or breathalyzer alcohol screen will be performed by a central laboratory.

e. The time of daily study drug dosing is to be recorded in the paper diary. Subjects will take study drugs as follows: once a day after dinner at day 1, twice a day within 2 hours after breakfast and dinner between day 2 and day 13 and once a day within 2 hours after breakfast at day 14. In order to collect the trough pharmacokinetic sample on day 14, the subject is to wait and take the morning dose at the site after pharmacokinetic sample collection has been performed.

f. Vital signs include blood pressure, heart rate and body temperature.

g. Specifically for the monitoring of creatinine, blood urea nitrogen (BUN), cystatin C and liver enzymes (renal and liver safety assessments) at visits 5 and 8.

h. At screening visit, subjects perform spirometry both pre and postbronchodilator, but only postbronchodilator thereafter: Subjects will receive 2 puffs of albuterol in the laboratory 10 to 15 minutes prior to the spirometry assessment on each study day.

i. At screening CWR will be completed at least 24 hours after IET at visit 2 and may be retested within the next 5 days at the investigator's discretion (within the visit window) if target exercise duration is not obtained. If this cannot be achieved with 2 adjustments (a total of 3 CWR tests), the subject will be classified as a screen failure and no additional assessments completed. All exercise tests (IET and CWR) will record heart rate and ECG simultaneously with breath-by-breath gas exchange and ventilatory variables during all exercise performance. Borg CR10 is performed with CWR at all of the time points shown in the schedule. The Electromyogram (EMG) is also performed with the CWR, but only at visits 4, 6, 7 and 9/EOT.

j. An accelerometer may be supplied to subjects after visit 2 due to the subject's availability between days -26 to -8.

k. Physical activity will be monitored for 7 continuous days during the screening period (baseline for physical activity), and treatment period 1 (endpoints). Subject will be given an accelerometer to wear during waking hours to monitor physical activity for 1 week. A weekly physical activity assessment (clinical visit version of PROactive physical activity in COPD [C-PPAC]) will be completed at the end of the 7-day activity monitoring using an accelerometer and a 7-day recall online 12-item questionnaire at the site.

l. Blood samples for pharmacokinetics of CK-2127107 and possible metabolite(s) (if applicable) will be collected from EVERY subject. A single pharmacokinetic sample will be collected at each of the following time points: prior to evening dose at day 1 of treatment period 2, prior to morning dose (trough) and then approximately 6 hours postmorning dose at day 14 of both treatment period 1 and 2.

m. Sample to be collected 1 time prior to first dose at day 1 of treatment period 1.

IS AMENDED TO:

Assessments	Screening				Treatment Period 1				Washout	Treatment Period 2			Follow-up
			~~Baseline for physical activity~~										
Day	-28 to -9	-27 to -8	~~-26 to -1~~[a]	-19 to -1	1	6	~~7 to 13~~	14	14 days	1	6	EOT[ba]/ED	7 days after EOT/ED
Window	-	-	-	-	-	+3	-	+3	+7	-	+3	+3	+7
Visit Number	1	2	-	3[eb]	4	5	-	6	-	7	8	9	10
Informed consent	X												
Inclusion/Exclusion	X												
Medical history	X				X					X			
Demographics	X												
Drug and alcohol screen[dc]	X				X					X			
Randomization					X								
Dispense/Collect					X			X		X		X	

study drug													
Administration of study drug[ed]					←				→		←		→
Physical examination[e]	X				X			X		X		X	X
Vital signs[f]	X				X			X		X		X	X
Hematology	X				X			X		X		X	X
Serum chemistries	X				X	X[g]		X		X	X[g]	X	X
Serology	X												
Urinalysis	X				X			X		X		X	X
Serum pregnancy test	X											X	X
Spirometry[h]	X				X			X		X		X	X
Respiratory muscle strength tests (MIP, MEP)					X			X		X		X	
12-lead ECG	X				X			X		X		X	X
CWR exercise test		X[i]			X			X		X		X	
QoL outcomes (SGRQ-C, SF-36)					X			X		X		X	
Physical performance test (SPPB)					X			X		X		X	
~~Leg extensor strength test (1RM)~~					~~X~~			~~X~~		~~X~~		~~X~~	
IET	X												
~~Supply accelerometer~~		~~X~~[j]				~~X~~							
~~Physical activity monitoring (Accelerometer and PRO)~~[k]			←		→		←	→					
~~Return accelerometer and complete~~ PRO **(C-PPAC)**				~~X~~	X			X					
Blood sampling for pharmacokinetics[ij]								Predose 6h		Predose		Predose 6h	
Optional blood sampling for pharmacogenomics[~~mk~~]					X								
Concomitant medication	X	X		X	X	X[l]	~~X~~	X	X	X	X[l]	X	X
Adverse events assessment	X	X		X	X	X[l]	~~X~~	X	X	X	X[l]	X	X

C-PPAC: clinical visit version of PRO active physical activity in COPD; CWR: constant work rate; ECG: electrocardiogram; ED: early discontinuation; EOT: end of treatment; IET: incremental exercise test; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; PRO: patient reported outcome; ~~1RM: 1 repetition maximum;~~ SF-36: Short Form-36; SGRQ-C: St. George's Respiratory Questionnaire for COPD; SPPB: short physical performance battery

~~a. Physical activity will be monitored at home for 7 days (1 week) and may occur between days -26 to -1. If the subject was not compliant or for some unforeseen reason the accelerometer did not record correctly, the physical activity monitoring and the assessment of a weekly PRO (7-day recall) may be repeated in the screening period.~~

~~b~~**a**. EOT: day 14 of treatment period 2**.**

~~c~~**b**. Visit 3 will be considered optional if the subject successfully completes the CWR at visit 2. Subjects who do not attend visit 3 will resume their visit schedule with visit 4.

~~d~~**c**. A standard urine screen for drugs of abuse (e.g., cannabinoids, cocaine and amphetamines) and benzodiazepines, and blood, urine, or breathalyzer alcohol screen will be performed by a central laboratory.

~~e~~**d**. The time of daily study drug dosing is to be recorded in the paper diary. Subjects will take study drugs as follows: once a day after dinner at day 1, twice a day within 2 hours after breakfast and dinner between day

2 and day 13 and once a day within 2 hours after breakfast at day 14. In order to collect the trough pharmacokinetic sample on day 14, the subject is to wait and take the morning dose at the site after pharmacokinetic sample collection has been performed.

e. A full physical examination will be performed at screening visit 1 and follow-up visit 10. Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal, and neuromuscular. At visits 4, 6, 7, and 9, a symptom-directed physical exam will be performed.

f. Vital signs include blood pressure, heart rate and body temperature.

g. Specifically for the monitoring of creatinine, blood urea nitrogen (BUN), cystatin C and liver enzymes (renal and liver safety assessments) at visits 5 and 8.

h. At screening visit, subjects perform spirometry both pre and postbronchodilator, but only postbronchodilator thereafter: Subjects will receive 2 puffs of albuterol in the laboratory 10 to 15 minutes prior to the spirometry assessment on each study day.

i. At screening CWR will be completed at least 24 hours after IET at visit 2 and may be retested within the next 5 days at the investigator's discretion (within the visit window) if target exercise duration is not obtained. If this cannot be achieved with 2 adjustments (a total of 3 CWR tests), the subject will be classified as a screen failure and no additional assessments completed. All exercise tests (IET and CWR) will record heart rate and ECG simultaneously with breath-by-breath gas exchange and ventilatory variables during all exercise performance. Borg CR10 is performed with CWR at all of the time points shown in the schedule. The Electromyogram (EMG) is also performed with the CWR, but only at visits 4, 6, 7 and 9/EOT.

~~j. An accelerometer may be supplied to subjects after visit 2 due to the subject's availability between days 26 to 8.~~

~~k. Physical activity will be monitored for 7 continuous days during the screening period (baseline for physical activity), and treatment period 1 (endpoints). Subject will be given an accelerometer to wear during waking hours to monitor physical activity for 1 week. A weekly physical activity assessment (clinical visit version of PROactive physical activity in COPD [C-PPAC]) will be completed at the end of the 7-day activity monitoring using an accelerometer and a 7-day recall online 12-item questionnaire at the site.~~

~~l~~**j**. Blood samples for pharmacokinetics of CK-2127107 and possible metabolite(s) (if applicable) will be collected from EVERY subject. A single pharmacokinetic sample will be collected at each of the following time points: prior to evening dose at day 1 of treatment period 2, prior to morning dose (trough) and then approximately 6 hours postmorning dose at day 14 of both treatment period 1 and 2.

~~m~~**k**. Sample to be collected 1 time prior to first dose at day 1 of treatment period 1.

l. If a subject experiences an AE or change in concomitant medications during days 7 to 13, they should call the study site to inform them of this change.

5 Treatments and Evaluation

5.3.1.4 Physical Activity

WAS:

At screening (visit 2) and treatment period 1 (visit 5) the subject will receive an accelerometer to be worn for 7 days between visit 2 and day-1 and days 7 to 13. Physical activity will be measured by wearing an accelerometer (movement intensity, different body positions, steps) for 7 days followed by an online 12 item PRO questionnaire (7 day recall [C PPAC]) which has to be completed at the site. Physical activity is assessed by questions targeted at the amount of physical activity and perceived difficulty during physical activity.

The European Union Innovative Medicines Initiative PROactive project [Gimeno-Santos et al, 2015] conducted qualitative research and drafted a conceptual framework to set out the concept of physical activity from patients' experience and to provide the necessary basis to

generate an item pool for 2 PROs, 1 with a daily recall period and 1 with a 7-day recall period (daily and clinical visit versions, respectively) to measure the experience of physical activity in patients with COPD.

Accelerometer: the DynaPort system (McRoberts BV [The Hague, The Netherlands]) requires that the monitor be initially programed with an activation time/date by physically attaching it to a computer with the DynaPort software. Monitors will stay attached to the charging station until they are administered to the subject to ensure a full 7 days of recording and instruct the subjects carefully how to wear the monitor and set it to "activated mode". Subjects are required to return to the clinic at the day 6 of treatment period 1 to be fitted with the physical activity monitor.

IS AMENDED TO:

~~At screening (visit 2) and treatment period 1 (visit 5) the subject will receive an accelerometer to be worn for 7 days between visit 2 and day 1 and days 7 to 13.~~ Physical activity will be measured ~~by wearing an accelerometer (movement intensity, different body positions, steps) for 7 days followed~~ by an online 12 item PRO questionnaire (7 day recall [C PPAC]) which has to be completed at the site. Physical activity is assessed by questions targeted at the amount of physical activity and perceived difficulty during physical activity.

The European Union Innovative Medicines Initiative PROactive project [Gimeno-Santos et al, 2015] conducted qualitative research and drafted a conceptual framework to set out the concept of physical activity from patients' experience and to provide the necessary basis to generate an item pool for 2 PROs, 1 with a daily recall period and 1 with a 7-day recall period (daily and clinical visit versions, respectively) to measure the experience of physical activity in patients with COPD.

~~Accelerometer: the DynaPort system (McRoberts BV [The Hague, The Netherlands]) requires that the monitor be initially programed with an activation time/date by physically attaching it to a computer with the DynaPort software. Monitors will stay attached to the charging station until they are administered to the subject to ensure a full 7 days of recording and instruct the subjects carefully how to wear the monitor and set it to "activated mode". Subjects are required to return to the clinic at the day 6 of treatment period 1 to be fitted with the physical activity monitor.~~

5 Treatments and Evaluation
5.3.2.5 IRM

DELETED

~~5.3.2.5 1RM~~

~~The leg extensor strength test, 1RM will be performed at treatment period 1 (visits 4 and 6) and treatment period 2 (visits 7 and 9). 1RM is a measure of the maximal weight a subject can lift with 1 repetition. 1RM is the heaviest weight a subject can lift once with seated leg press, which largely reflects activation of the quadriceps muscles, hamstrings and gluteus maximus.~~

5 Treatments and Evaluation
5.4.4 Physical Examination

WAS:

A physical examination will be performed at the screening and at the visits 4, 6, 7, 8, 9 and 10. Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular.

Only the date of the physical examination will be recorded in the eCRF. Any clinically relevant abnormality at screening will be recorded as Medical History. Any clinically relevant change from baseline to visit 9/EOT will be recorded as an AE in the eCRF.

IS AMENDED TO:

A full physical examination will be performed at screening visit 1 and follow-up visit 10. Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal, and neuromuscular. At the visits 4, 6, 7, and 9, a symptom-directed physical exam will be performed.

~~Only the date of the physical examination will be recorded in the eCRF.~~ Any clinically relevant abnormality at screening will be recorded as medical history. Any clinically relevant change from baseline to visit ~~9/EOT~~ **10/FUV** will be recorded as an AE in the eCRF. **Documentation that an exam has occurred needs to be reflected in the site source documents.**

Screening physical examination is to be conducted in person by a physician. Remaining physical examinations can be symptom directed and delegated to a Nurse Practitioner or Physician Assistant (if the study physician is unavailable) and signed off by the study physician. ~~A physical examination will be performed at the screening and at the visits 4, 6, 7, 8, 9 and 10. Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular.~~

~~Only the date of the physical examination will be recorded in the eCRF. Any clinically relevant abnormality at screening will be recorded as Medical History. Any clinically relevant change from baseline to visit 9/EOT will be recorded as an AE in the eCRF.~~

5 Treatments and Evaluation
5.8 Total Amount of Blood

WAS:

- Approximately 5 mL will be collected for pharmacogenetics testing (optional).

IS AMENDED TO:

- Approximately **4**~~5~~ mL will be collected for pharmacogenetics testing (optional).

7 Statistical Methodology *7.1 Sample Size*
WAS:
A sample size of 40 subjects will provide 84% power to detect a difference of 60 seconds in CWR duration from period baseline for CK-2127107 versus placebo with a 1-sided alpha error of 0.05, assuming the standard deviation for change from period baseline is 100 seconds. In order to maintain the statistical power of 80%, approximately 36 subjects would need to complete period 1. Should the discontinuation rate in the first treatment period exceed 10%, additional subjects will need to be recruited.
IS AMENDED TO:
A sample size of 40 subjects will provide 84% power to detect a difference of 60 seconds in CWR duration from period baseline for CK-2127107 versus placebo with a 1-sided alpha error of 0.05, assuming the standard deviation for change from period baseline is 100 seconds. In order to maintain the statistical power of 80%, approximately 36 subjects would need to complete period 1. Should the discontinuation rate in the first treatment period exceed 10% **(approximately 5 subjects)**, additional subjects will need to be recruited **to account for the discontinued subjects**.

7 Statistical Methodology *7.2.1 Full Analysis Set (FAS)*
WAS:
7.2.1 Full Analysis Set (FAS) The full analysis set (FAS) will consist of all subjects who are randomized and receive at least 1 dose of study drug and have at least 1 baseline measurement and 1 post baseline measurement within a period. Subjects will be included in the treatment arm based on randomization regardless of actual treatment received. This will be the primary analysis set for efficacy analyses.
IS AMENDED TO:
7.2.1 Full Analysis Set ~~(FAS)~~ The full analysis set (FAS) will consist of all subjects who are randomized and receive at least 1 dose of study drug and have at least 1 baseline measurement and 1 post baseline measurement within a period. Subjects will be included in the treatment arm based on ~~randomization regardless of~~ actual treatment received. This will be the primary analysis set for efficacy analyses.

III. Non-Substantial Amendment Rationale:

Rationale for Non-Substantial Designation
All revisions made to the protocol are administrative in nature and do not impact the safety or scientific value of the clinical study.

14 SPONSOR'S SIGNATURES