RTA 408

408-C-1403

NCT02255422

A PHASE 2 STUDY OF THE SAFETY, EFFICACY, AND PHARMACODYNAMICS OF RTA 408 IN THE TREATMENT OF MITOCHONDRIAL MYOPATHY

VERSION 5.0 – 29 OCTOBER 2015

SPONSOR APPROVAL AND SIGNATURE PAGE

[redacted]

Date

[redacted]

Date

[redacted]

Date

INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure for RTA 408. I have read the 408-C-1403 clinical study protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

Role in Study	Name	Address and Telephone Number
Clinical Study Manager	[redacted]	[redacted]
Medical Monitor	[redacted]	[redacted]
Serious Adverse Event (SAE) Reporting	[redacted]	

2. SYNOPSIS

Name of Sponsor/Company:
Reata Pharmaceuticals, Inc.

Name of Investigational Products:
RTA 408 Capsules

Name of Active Ingredient:
RTA 408

Title of Study:
A Phase 2 Study of the Safety, Efficacy, and Pharmacodynamics of RTA 408 in the Treatment of Mitochondrial Myopathy

Study center(s): Up to 12 study centers in the United States and the European Union

Studied period (years): Approximately 2 years Estimated date first patient enrolled: November 2014 Estimated date last patient completed: December 2016	**Phase of development:** 2

Objectives:
In patients with mitochondrial myopathy, comparing those receiving RTA 408 versus those receiving the placebo, the objectives are as follows:

Primary:

- To evaluate the change in peak work during maximal exercise testing
- To evaluate the safety and tolerability of RTA 408

Secondary:

- To evaluate the change in 6-minute walk test (6MWT) distance

Exploratory:

- To evaluate the change in peak oxygen utilization during maximal exercise testing
- To evaluate the change in peak serum lactate and pyruvate, peak heart rate, and rating of perceived exertion during submaximal exercise testing
- [redacted]
- [redacted]
- [redacted]

Methodology:
This 2-part study will evaluate the efficacy, safety, and pharmacodynamics (PD) of RTA 408 in the treatment of patients with mitochondrial myopathy.

Part 1: The first part of the study will be a randomized, placebo-controlled, double-blind, dose-ranging study to evaluate the safety, efficacy, and pharmacodynamic (PD) activity of RTA 408 at 2.5 mg, 5 mg, 10 mg, 20 mg and higher dose levels (not to exceed 160 mg) in patients with mitochondrial myopathy. A cohort consists of the next eight eligible patients randomized 3:1 at the cohort specific dose (n=6) or

placebo (n=2). Approximately 8 cohorts will be enrolled in Part 1 to allow for adequate dose-ranging for selection of two doses of RTA 408 to be used in Part 2.

Intra-patient dose-escalation will only be utilized in the first cohort to evaluate RTA 408 at the first two dose levels (2.5 mg and 5 mg). Patients enrolling in the first cohort will be randomized to RTA 408 2.5 mg or placebo. After the Week 2 visit, each patient in the first cohort will dose escalate to 5 mg (or remain on placebo) on Day 15 unless a dose-limiting toxicity (DLT) is reported in that patient (Section 7.4.3). After the last patient in the first cohort completes their Week 4 visit (i.e., 2 weeks on 2.5 mg daily [or matching placebo] followed by 2 weeks on 5 mg daily [or matching placebo]), the data safety monitoring board (DSMB) and Sponsor will review all available safety information and make a decision regarding enrollment of the next cohort Beginning with the second 8-patient cohort, once the eighth patient enrolled completes their Week 2 visit the DSMB will review all available safety information and recommend the dose of RTA 408 for the subsequent cohort. The DSMB dose recommendation for each cohort must not exceed 100% more than the highest dose of RTA 408 evaluated in this study, and the maximum permitted dose of RTA 408 is 160 mg. The dose-level for each new cohort will not exceed the DSMB recommended dose level, and it will be selected by the Sponsor based on review of available safety, efficacy, PK, and PD data. Prior to opening each cohort in Part 1 for enrollment, the Sponsor will evaluate all available data from doses studied in Part 1 to determine if enough information is available to select doses for Part 2 of the study. Once doses are selected for Part 2 by the Sponsor, no additional cohorts will be enrolled in Part 1

Part 2: The second part of this study will be a randomized, placebo-controlled, double-blind, parallel study to evaluate the safety, efficacy, and PD of 2 dose levels of RTA 408 in patients with mitochondrial myopathy. Assuming that doses tested in Part 1 have been deemed acceptable in the first part of the study, the first 24 eligible patients in Part 2 will be randomized 1:1:1 to receive RTA 408 at the selected doses or placebo (n=8 per treatment group). The two doses to be selected are based on DSMB and Sponsor review of data from Part 1, including safety and available PK and PD data. Patients will be stratified by peak work at baseline (>0.8 watts [W]/kg vs. ≤0.8 W/kg). A blinded sample size recalculation will occur after the last patient is enrolled in Part 2 to evaluate the distribution of baseline peak work for all enrolled patients as described in Section 14.1.1 and assess the need for additional patients. If the sample size recalculation demonstrates additional patients are needed, then expansion of the number of patients enrolled in Part 2 will occur and an additional 12 patients may be randomized, for a total of 36 patients enrolled in Part 2 (n=12 per treatment group).

All qualified patients enrolled in the study (i.e., both Part 1 and Part 2) will follow nearly identical schedules of assessments (Table 3) and study drug administration. Patients will self-administer study treatment once daily at the randomized dose level until they have completed 12 weeks (84 days) of study treatment, are discontinued from study treatment, or have withdrawn consent to participate in the study. A follow-up visit for safety will occur at Week 16 (4 weeks after last dose). The DSMB will perform monthly reviews of unblinded data for safety throughout the study (i.e., during both Part 1 and Part 2).

Number of patients (planned):

Approximately 100 patients will be enrolled in this study.

Inclusion criteria:

Patients must:

1. Have mitochondrial myopathy as evidenced by the following 2 criteria (must meet both):
    a. Have a history of exercise intolerance with or without weakness and/or progressive exercise intolerance (in which modest exercise typically provokes heaviness, weakness, aching of active muscles, or tachycardia)
    b. Have a known primary mitochondrial DNA mutation or a nuclear DNA defect that is associated with reduced activity of at least 1 mitochondrially encoded respiratory chain complex
2. Be male or female and ≥18 years of age and ≤75 years of age
3. Have no changes to their exercise regimen within 30 days prior to Study Day 1 and be willing to remain on the same exercise regimen during the 16-week study period
4. Have the ability to complete maximal exercise testing
5. Have peak work during maximal exercise testing of ≤1.5 W/kg
6. Have adequate kidney function defined as an estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease (MDRD) 4-variable formula
7. Be able to swallow capsules
8. Be willing and able to cooperate with all aspects of the protocol
9. Be willing to practice the medically acceptable methods of birth control (Section 9.7.2)
10. Provide written informed consent for study participation, approved by the appropriate Institutional Review Board or Ethics Committee

Exclusion criteria:

Patients must not:

1. Have uncontrolled diabetes (HbA1c >11.0%)
2. Have B-type natriuretic peptide level >200 pg/mL
3. Have a history of clinically significant left-sided heart disease and/or clinically significant cardiac disease, including but not limited to any of the following:
    a. Clinically significant congenital or acquired valvular disease
    b. Pericardial constriction (based on echocardiogram performed at Screening Visit or within 30 days prior to Screening Visit)
    c. Restrictive or congestive cardiomyopathy (based on echocardiogram performed at Screening Visit or within 30 days prior to Screening Visit)
    d. Symptomatic coronary disease (prior myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft surgery, or angina)
    e. Evidence of left ventricular diastolic dysfunction
    f. History of atrial fibrillation
    g. History of unstable arrhythmias
    h. Cardiac insufficiency, defined as New York Heart Association Class >2
4. Have known active fungal, bacterial, and/or viral infection, including human immunodeficiency virus or hepatitis virus (B or C)
5. Have known or suspected active drug or alcohol abuse, as per investigator judgment
6. Have clinically significant abnormalities of clinical hematology or biochemistry, including but

not limited to elevations greater than 1.5 times the upper limit of normal of aspartate aminotransferase, alanine aminotransferase or creatinine. Levels above this threshold are allowable if attributable to muscle injury.

7. Have any abnormal laboratory test value or clinically significant pre-existing medical condition that, in the opinion of the investigator, would put the patient at risk by study enrollment
8. Have taken any of the following drugs within 7 days prior to Study Day 1 or plan to take any of these drugs during the time of study participation:
    a. Sensitive substrates for cytochrome P450 2C8 or 3A4 (e.g., repaglinide, midazolam, sildenafil)
    b. Moderate or strong inhibitors or inducers of cytochrome P450 3A4 (e.g., carbamazepine, phenytoin, ciprofloxacin, grapefruit juice)
    c. Substrates for p-glycoprotein transporter (e.g., ambrisentan, digoxin)
9. Have a history of clinically significant liver disease (e.g., fibrosis, cirrhosis, hepatitis), or has, at screening, clinically relevant deviations in laboratory tests including any one of the following:
    a. alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) > 1.5-fold ULN,
    b. bilirubin > 1.2-fold ULN,
    c. alkaline phosphatase (ALP) > 2-fold ULN,
    d. kidney insufficiency as defined by creatinine level > 1.5 mg/dL,
    e. albumin < lower limit of normal (LLN)
10. Have participated in any other interventional clinical study within 30 days prior to Study Day 1
11. Have a cognitive impairment that may preclude ability to comply with study procedures
12. Be unable to comply with the requirements of the study protocol or be unsuitable for the study for any reason, in the opinion of the investigator
13. Have used antioxidant supplements, including but not limited to idebenone, coenzyme Q10, nicotinamide, and vitamin E above the recommended daily allowance, within 14 days prior to Study Day 1 or plan to take any of these supplements during the time of study participation
14. Have taken chronic treatment with systemic corticosteroids within 30 days prior to Study Day 1
15. Have had significant suicidal ideation within 1 month prior to Screening Visit as per investigator judgment or any history of suicide attempts
16. Be pregnant or breastfeeding

Investigational product, dosage and mode of administration:

RTA 408 Capsules (2.5 mg, 10 mg or 50 mg) administered orally with placebos, as required per treatment assignment

Duration of treatment:

RTA 408 or placebo capsules administered orally once daily for 12 weeks

Reference therapy, dosage, and mode of administration:

This study does not employ a comparator drug.

Endpoints:

Efficacy: Parameters collected during maximal exercise testing (including peak work and peak oxygen utilization) and submaximal exercise testing; 6MWT distance; Fatigue Severity Scale results; and SF-36 test results

Safety: Results of echocardiogram, electrocardiogram, vital sign measurements, weight, body mass index, physical examinations, adverse events, serious adverse events, concomitant medications, and laboratory test results (clinical chemistry, hematology, urinalysis, microscopy, and pregnancy tests [as indicated])

Pharmacokinetic: RTA 408 plasma concentration data

Pharmacodynamic: Parameters assessed from serum lactate and serum pyruvate during submaximal exercise testing, muscle needle biopsy biomarkers.

Statistical methods:

Sample size: The sample size for Part 1 is based on a dose-escalation scheme to evaluate initial safety and PD activity of RTA 408 in this patient population. The small number of patients at each dose in Part 1 is not expected to fully characterize safety, efficacy, or PD, but rather inform the DSMB and Sponsor of the appropriate doses to select for Part 2. With 24 patients enrolled in Part 2 (8 patients per treatment cohort), this study has approximately 80% power to detect an improvement of 0.28 W/kg in the peak work of pooled RTA 408 (n=16) versus placebo (n=8) assuming a two-sided Type I error rate of 0.05, a common within-group standard deviation (SD) of 0.28 W/kg, and a drop-out rate of no more than 1 in the placebo group and no more than 2 in the pooled RTA 408 group. The analysis will use repeated measures methodology, assuming compound symmetry for the within-subject covariance structure (ρ=0.20).

Sample size recalculation: A sample size recalculation will be performed in Part 2 after all 24 patients have been randomized to evaluate the pooled distribution of baseline peak work. If the SD for pooled, baseline peak work is greater than 0.28 W/kg or the distribution is not approximately normal, then an additional 12 patients will be enrolled in Part 2. With 36 patients enrolled in Part 2 (i.e., 12 patients per randomized treatment cohort), the 80% power is retained to detect the same difference of 0.28 W/kg if the SD is up to 0.35 W/kg, under the same assumptions of a two-sided Type I error rate of 0.05, and a drop-out rate of no more than 1 in the placebo group and no more than 2 in the pooled RTA 408 group, assuming compound symmetry for the within-subject covariance structure (ρ=0.20).

Statistical analysis: A statistical analysis plan (SAP) detailing the analyses to be performed will be developed prior to the database lock. The SAP, which will describe in detail the methods used for the primary and secondary endpoints, will serve as the final arbiter of all statistical analyses. Data will be summarized using descriptive statistics. Continuous data will be summarized with number of patients (n), mean, median, minimum, maximum, SD, coefficient of variation, and geometric mean (where applicable). Categorical data will be summarized using frequency counts and percentages.

Primary efficacy analysis: Primary analysis of the efficacy data will be based on the modified intent-to-treat population, which will include all patients enrolled in Part 2 of the study. Peak work from Part 2 for the pooled patients treated with RTA 408 (both dose levels will be compared with placebo after 12 weeks of treatment by repeated measures analysis of covariance, with treatment group, time, and the interaction between treatment and time as fixed factors and baseline peak work as a covariate. The difference between RTA 408 and placebo in change from baseline of mean peak work will be estimated along with the 95% confidence interval of the difference. The SAP will describe the strategy used to control the Type I error rate when comparing each dose of RTA 408 with placebo.

3. TABLE OF CONTENTS AND LISTS OF TABLES AND FIGURES

LIST OF TABLES

LIST OF FIGURES

4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations are used in this study protocol.

Table 2: List of Abbreviations

Abbreviation	Explanation
6MWT	6-minute walk test
AE	adverse event
ALP	alkaline phosphatase
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the plasma drug concentration-time curve
BMI	body mass index
BNP	B-type natriuretic peptide
BUN	blood urea nitrogen
CDDO	2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid
CDDO-EA	2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid ethylamide
CDDO-Im	2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid imidazole
CDDO-Me	2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid methyl ester
CDDO-TFEA	2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid trifluoroethylamide
CFR	Code of Federal Regulations
C_{max}	maximum analyte concentration in plasma
COX	cytochrome *c* oxidase
CPK	creatine phosphokinase
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose-limiting toxicity
DHMA	Danish Health and Medicines Authority
DSMB	data safety monitoring board
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic case report form

Abbreviation	Explanation
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
FDA	Food and Drug Administration
FGF21	fibroblast growth factor 21
GCP	Good Clinical Practice
GGT	gamma-glutamyl transpeptidase
GLP	Good Laboratory Practice
GSTA3	glutathione S-transferase A3
HDL-C	high-density lipoprotein cholesterol
HDPE	high-density polyethylene
HIV	human immunodeficiency virus
HO-1	heme oxygenase-1
ICF	informed consent form
ICH E6(R1)	International Conference on Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice E6(R1)
IFNγ	interferon-gamma
IRB	Institutional Review Board
ITT	intent-to-treat
IWRS	Interactive Web Response System
Keap1MuKO	Keap1 gene hypomorphic knockdown in mice
LDH	lactate dehydrogenase
LDL-C	low-density lipoprotein cholesterol
LLN	lower limit of normal
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
mtDNA	mitochondrial DNA
Na_2SO_3	sodium sulfite
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells

Abbreviation	Explanation
NO	nitric oxide
NOAEL	no-observed-adverse-effect level
NQO1	NAD(P)H dehydrogenase, quinone 1
Nrf2	nuclear factor erythroid-derived 2-related factor 2
NT-proBNP	N-terminal prohormone of B-type natriuretic peptide
PD	pharmacodynamic(s)
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PK	pharmacokinetic(s)
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SF-36	SF-36® Health Survey Update
SOD1	superoxide dismutase 1
SpO_2	pulse oximetry oxygen saturation
T_{max}	time to maximum analyte concentration in plasma
ULN	upper limit of normal
US	United States
US CFR Title 21	Title 21 of the US Code of Federal Regulations
VLDL-C	very-low-density lipoprotein cholesterol
W	watts
WBC	white blood cell
WOCBP	women of childbearing potential

5. INTRODUCTION

5.1. Background and Rationale for RTA 408 in Mitochondrial Myopathy

Mitochondrial myopathies are a multisystemic group of disorders that are characterized by a wide range of biochemical and genetic mitochondrial defects and variable modes of inheritance. Convoluted mitochondrial DNA (mtDNA) tissue heteroplasmy, the lack of a clear genotype, and the complex interactions between the nuclear and mitochondrial genome complicate the ability to predict a disease course and to classify the type of myopathy. Indeed, the lack of a clear disease phenotype has frustrated treatment efforts, and currently there are no effective treatments for this disease (Goldstein and Wolfe, 2013). Despite the heterogeneous myopathy phenotypes, a unifying feature of mitochondrial myopathies is that the pathogenic mtDNA mutations and/or nuclear mutations of the electron transport chain invariably lead to dysfunctional mitochondrial respiration. This reduction in mitochondrial respiration leads to a reduced ability to produce cellular adenosine triphosphate (ATP), often resulting in muscle weakness, exercise intolerance, and fatigue in patients with mitochondrial myopathy.

Although no approved treatments exist for mitochondrial myopathy, several studies have demonstrated that exercise training increases mitochondrial biogenesis in patients with mitochondrial myopathy (Taivassalo, 2001; Cejudo, 2005; Taivassalo, 2006; Adhihetty, 2007). Furthermore, the increases in mitochondrial biogenesis in these patients led to improvements in maximal workload and oxygen consumption, as well as a reduction in plasma lactate during exercise (Taivassalo, 2006; Adhihetty, 2007). Previous studies have demonstrated that exercise stress activates nuclear factor erythroid-derived 2-related factor 2 (Nrf2) signaling and promotes antioxidant mechanisms (George, 2008; Irving, 2011; Muthusamy, 2012); therefore, Nrf2-dependent mechanisms may contribute to exercise improvements in patients with mitochondrial myopathy.

Several lines of evidence suggest that Nrf2 activation can increase mitochondrial respiration and biogenesis. First, activation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in a mouse model of mitochondrial myopathy showed increased mitochondrial mass and improved ATP production. These results indicated that activation of PGC-1α, an Nrf2-target gene, led to increased mitochondrial biogenesis (Wenz, 2008). In addition, Uruno *et al*. (2013) demonstrated that genetic activation of Nrf2 signaling by Keap1 gene hypomorphic knockdown in mice (Keap1MuKO) markedly increased energy consumption-related genes in skeletal muscle. Genetic Nrf2 activation led to increased locomotor activity, as compared to control (Keap1F/F) mice. Locomotor activity was increased as determined in an open-field test and quantitated in terms of total distance traveled, as well as the number of zones crossed during the testing period (0 to 20 min). These results suggest that activation of Nrf2 in skeletal muscle results in locomotor improvement due to increased efficiency of oxidative phosphorylation.

Moreover, 2 recent studies demonstrated that genetic Nrf2 activation in Keap1-knockout mice increased mitochondrial respiration, oxygen consumption, and ATP production compared with Nrf2-deficient mice (Holmström, 2013; Ludtmann, 2014). In essence, Nrf2 activation increased substrate availability for mitochondrial respiration, which led to improvements in ATP production.

Natural triterpenoids, such as oleanolic acid and ursolic acid that are derived from plant extracts, have been used extensively in Asian medicine for their antioxidant, anti-inflammatory, and anti-cancer properties (Liu, 1995). RTA 408 is one of a class of semi-synthetic triterpenoids discovered through a medicinal chemistry effort to optimize the ability of these compounds to inhibit the induction of nitric oxide (NO) in primary mouse macrophages treated with interferon-gamma (IFNγ; Honda, 1999). Subsequent mechanistic studies have revealed that RTA 408 and the semi-synthetic triterpenoids are potent activators of Nrf2 and inhibitors of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), and thus induce an antioxidant and anti-inflammatory phenotype.

Several preclinical studies have highlighted the beneficial effects of RTA 408 analogs on muscle function, oxidative phosphorylation, and mitochondrial biogenesis. Shin *et al.* (2009) demonstrated that potent Nrf2 activation with a 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO)-imidazolide (CDDO-Im) triterpenoid prevented high-fat diet-induced increases in body weight, adipose mass, and hepatic lipid accumulation in wild-type mice. Wild-type mice on a high-fat diet and treated with CDDO-Im exhibited higher oxygen consumption and energy expenditure than vehicle-treated mice, while food intake was lower in CDDO-Im-treated than vehicle-treated mice. These results highlight the ability of potent Nrf2 activation to increase mitochondrial respiration.

Saha *et al*. (2010) demonstrated that in high-fat diet–fed type 2 diabetic mice, oral treatment with bardoxolone methyl, an RTA 408 analog (also referred to as CDDO-methyl ester [CDDO-Me]), for 2 weeks improved glucose uptake, fatty acid oxidation, and oxygen consumption. Specifically, treatment with bardoxolone methyl led to a 48% increase in the mean glucose disposal rate, reflecting increased peripheral insulin responsiveness induced by bardoxolone methyl. The bardoxolone methyl treatment also increased insulin-stimulated glucose transport activity in skeletal muscles by 71% in the soleus and by 58% in the gastrocnemius. These results demonstrate that the glucose-lowering activity of bardoxolone methyl results from enhanced insulin action and glucose delivery to the muscles, which was associated with an increased rate of oxidative phosphorylation within the skeletal muscle.

Furthermore, Neymotin *et al*. (2011) examined 2 additional RTA 408 analogs, CDDO ethylamide (CDDO-EA) and CDDO-trifluoroethylamide (CDDO-TFEA), that potently activate Nrf2 in the G93A superoxide dismutase 1 (SOD1) mouse model of amyotrophic lateral sclerosis (ALS). CDDO-EA and CDDO-TFEA significantly induced the mRNA expression of the Nrf2 target genes NAD(P)H dehydrogenase, quinone 1 (NQO1), glutathione S-transferase A3 (GSTA3), and heme oxygenase-1 (HO-1) relative to controls while suppressing pro-inflammatory NF-κB target genes. Moreover, genes associated with increased mitochondrial biogenesis were also induced. Collectively, the data suggest that the ability of RTA 408 to activate Nrf2 and induce its target genes could potentially improve muscle function, oxidative phosphorylation, antioxidant capacity, and mitochondrial biogenesis in patients with mitochondrial myopathy.

Regarding the safety profile of RTA 408, no adverse effects were observed in the safety pharmacology studies. The genotoxicity potential of RTA 408 was investigated in 2 *in vitro* genetic toxicity tests and 2 *in vivo* genotoxicity studies in rats. The overall weight of evidence from the genotoxicity studies indicates that RTA 408 has a low genotoxicity risk to human subjects.

The systemic toxicity potential of RTA 408 has been evaluated in multiple nonclinical Good Laboratory Practice (GLP) toxicity studies in rats, minipigs, and monkeys after oral and dermal (which produces meaningful systemic exposure to RTA 408 in animals) administration. The primary target organs for RTA 408 observed in the rat and monkey include the liver (increased liver weight, hepatocellular hypertrophy, bile duct hypertrophy and hyperplasia) and kidney (tubular degeneration/regeneration). These effects are considered to be mostly due to the known pharmacologic activity of RTA 408 in animals and likely do not reflect off-target toxicity. In rats (but not monkeys or minipigs), minimal to mild squamous cell hyperplasia of the forestomach and/or the limiting ridge of the stomach was observed and was fully reversible on treatment discontinuation. The forestomach and limiting ridge of the stomach do not exist in humans, a monogastric species. Therefore, this finding does not translate to humans and is not considered a relevant risk to humans.

Overall, rats are more sensitive to the toxicologic effects of RTA 408 than minipigs or monkeys. In rats, the no-observed-adverse-effect level (NOAEL) following 28 days or 13 weeks or daily oral administration was 3 mg/kg/day, corresponding to an area under the plasma drug concentration-time curve $(AUC)_{(0\text{-}24hr)}$ of approximately 2.0 hr*µg/mL. Following 6 months of oral administration to rats, adverse effects were observed at the lowest dose tested (0.3 mg/kg), which was associated with an $AUC_{(0\text{-}24hr)}$ of approximately 0.3 hr*µg/mL. Similar systemic exposure was achieved in rats at the lowest dose tested in a 13-week topical dermal toxicity study of RTA 408 Lotion, with similar adverse effects observed in males, but not females, in the low-dose group. In monkeys, the NOAEL following 28 days or 13 weeks of daily oral administration was at least 100 mg/kg/day and the NOAEL following 9 months of daily oral administration was 30 mg/kg/day, corresponding to an $AUC_{(0\text{-}24hr)}$ of approximately 2.0 hr*µg/mL. In a 13-week minipig dermal GLP toxicity study, the NOAEL was the maximal feasible dose of 8% RTA 408 Lotion (twice daily topical administration) with systemic exposures up to 1.7 hr*µg/mL.

Across studies, the adverse liver findings in rats were reversible upon drug discontinuation and were associated with moderate to marked increases in serum gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin. With short treatment duration (i.e., 14 days), tubular degeneration/regeneration in rats was reversible with a 28-day treatment-free period in a non-GLP study. Although the kidney findings in rats after 28 days or longer of treatment were not reversible after a 4-week or 8-week recovery period, they were minimal to mild, were not associated with evidence of an effect on renal function (i.e., no increase in blood urea nitrogen [BUN] or serum creatinine), and were not present in minipigs or monkeys with dosing for up to 13 weeks (minipig dermal) or 9 months (monkey oral) at doses that produced systemic exposures approximately 10-fold above the exposures that elicited adverse kidney findings in rats.

6. STUDY OBJECTIVES AND PURPOSE

In patients with mitochondrial myopathy, comparing those receiving RTA 408 versus those receiving placebo, the objectives are as follows:

6.1. Primary Objectives

- To evaluate the change in peak work during maximal exercise testing
- To evaluate the safety and tolerability of RTA 408

6.2. Secondary Objective

- To evaluate the change in 6-minute walk test (6MWT) distance

6.3. Exploratory Objectives

- To evaluate the change in peak oxygen utilization during maximal exercise testing
- To evaluate the change in peak serum lactate and pyruvate, peak heart rate, and rating of perceived exertion during submaximal exercise testing
- [redacted]

7. INVESTIGATIONAL PLAN

7.1. Overall Study Design

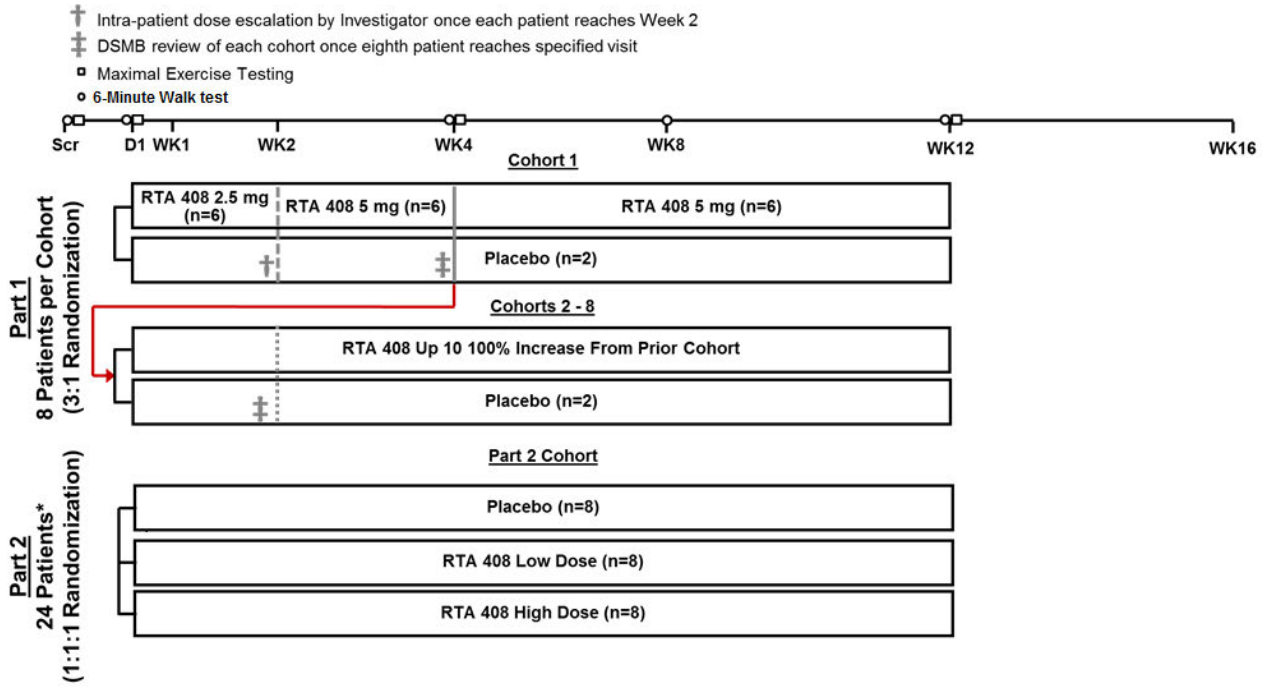
This 2-part study will evaluate the efficacy, safety, and PD of RTA 408 in the treatment of patients with mitochondrial myopathy.

Part 1: The first part of the study will be a randomized, placebo-controlled, double-blind, dose-ranging study to evaluate the safety, efficacy, and pharmacodynamic (PD) activity of RTA 408 at 2.5 mg, 5 mg, 10 mg, 20 mg and higher dose levels (not to exceed 160 mg) in patients with mitochondrial myopathy. A cohort consists of the next eight eligible patients randomized 3:1 to RTA 408 at the cohort specific dose (n=6) or placebo (n=2). Approximately 8 cohorts will be enrolled in Part 1 of the study to allow for adequate dose-ranging for selection of two doses of RTA 408 to be used in Part 2.

Intra-patient dose-escalation will only be utilized in the first cohort to evaluate RTA 408 at the first two dose levels (2.5 mg and 5 mg). Patients enrolling in the first cohort will be randomized to RTA 408 2.5 mg or placebo. After the Week 2 visit, each patient in the first cohort will dose escalate to 5 mg (or remain on placebo) on Day 15 unless a dose-limiting toxicity (DLT) is reported in that patient (Section 7.4.3). After the last patient in the first cohort completes their Week 4 visit (i.e., 2 weeks on 2.5 mg daily [or matching placebo] followed by 2 weeks on 5 mg daily [or matching placebo]), the data safety monitoring board (DSMB) and Sponsor will review all available safety information and make a decision regarding enrollment of the next cohort. Beginning with the second 8-patient cohort, once the eighth patient enrolled completes their Week 2 visit, the DSMB will review all available safety information and recommend the dose of RTA 408 for the subsequent cohort. The DSMB dose recommendation for each cohort must not exceed 100% more than the highest dose of RTA 408 evaluated in this study, and the maximum permitted dose of RTA 408 is 160 mg. The dose-level for each new cohort will not exceed the DSMB recommended dose level, and it will be selected by the Sponsor based on review of available safety, efficacy, PK, and PD data. Prior to opening each cohort in Part 1 for enrollment, the Sponsor will evaluate all available data from doses studied in Part 1 to determine if enough information is available to select doses for Part 2 of the study. Once doses are selected for Part 2 by the Sponsor, no additional cohorts will be enrolled in Part 1.

Part 2: The second part of this study will be a randomized, placebo-controlled, double-blind, parallel study to evaluate the safety, efficacy, and PD of 2 dose levels of RTA 408 in patients with mitochondrial myopathy. Assuming that the doses tested in Part 1 have been deemed acceptable in the first part of the study, the first 24 eligible patients in Part 2 will be randomized 1:1:1 to receive RTA 408 at the selected doses , or placebo (n=8 per treatment group). The two doses to be selected are based on DSMB and Sponsor review of data from Part 1, including available safety and PK and PD data. Patients will be stratified by peak work at baseline (>0.8 watts [W]/kg vs. ≤0.8 W/kg). A blinded sample size recalculation will occur after the last patient is enrolled in Part 2 to evaluate the distribution of baseline peak work for all enrolled patients as described in Section 14.1.1 and assess the need for additional patients. If the sample size recalculation demonstrates additional patients are needed, then expansion of the number of patients enrolled in Part 2 will occur and an additional 12 patients may be randomized, for a total of 36 patients enrolled in Part 2 (n=12 per treatment group).

Figure 1: Schema for Study of RTA 408 in Patients with Mitochondrial Myopathy



*A blinded sample size recalculation may increase the sample size to 12 patients per treatment group in Part 2.

A schema for the protocol is shown in Figure 1 and a detailed schedule of assessments for the study is shown in Table 3. All qualified patients enrolled in the study (i.e., both Part 1 and Part 2) will follow nearly identical schedules of assessments (Table 3) and study drug administration. Patients will self-administer study treatment once daily until they have completed 12 weeks (84 days) of study treatment, are discontinued from study treatment, or have withdrawn consent to participate in the study. A follow-up visit for safety will occur at Week 16 (4 weeks after last dose). The DSMB will perform monthly reviews of unblinded data for safety throughout the study (i.e., during both Part 1 and Part 2).

7.2. Number of Patients

Approximately 100 patients will be enrolled in this study.

7.3. Treatment Assignment

Patients enrolled in the first cohort for Part 1 will be randomized 3:1 to receive RTA 408 2.5 mg or placebo. After the Week 2 visit, patients receiving RTA 408 2.5 mg will dose escalate to RTA 408 5 mg starting on Day 15 unless a DLT is observed. Intra-patient dose-escalation will only be utilized in the first cohort for Part 1. Starting with the second cohort for Part 1, patients will be randomized 3:1 to receive the cohort specific dose level of RTA 408 or placebo and will remain on the same treatment assignment throughout the study. . Two dose levels of RTA 408 will be evaluated in Part 1 will be used in Part 2. Patients enrolled in Part 2 of the study will be randomized 1:1:1 to RTA 408 low dose, high dose, or placebo. All randomizations will be

performed using an Interactive Web Response System (IWRS). Patients enrolled in Part 2 will be stratified by peak work at baseline (>0.8 W/kg vs. ≤0.8 W/kg).

7.4. Dose Selection and Escalation Scheme

7.4.1. Selection of Doses

This 2-part study will evaluate the safety, efficacy, and PD of RTA 408 at several dose levels (2.5 mg, 5 mg, 10 mg, 20 mg, and higher doses not to exceed 160 mg) in the treatment of patients with mitochondrial myopathy.

[redacted] This observation is consistent with the fact that plasma RTA 408 concentrations achieved at the 2.5-mg dose level are above those required for *in vitro* activation of Nrf2. Dose escalation by increments up to 100% increase from the each prior cohort, to a maximum dose of 160 mg, was selected as the highest potential dose in this study in an effort to adequately dose range and to provide good separation of treatment effects between dose groups. Doses from Part 1 will be evaluated for safety, efficacy, and PD to select two doses which appear to be pharmacologically active in patients with mitochondrial myopathy to be used in Part 2.

7.4.2. Dose-Escalation Scheme

In Part 1 of the study, an initial cohort of 8 patients will be randomized 3:1 to RTA 408 2.5 mg (n=6) or placebo (n=2). Each patient in the first cohort will be evaluated for intra-patient dose-escalation by their investigator at the Week 2 visit. If no DLTs are observed by Week 2 for that patient, the patient will dose escalate. The patients receiving RTA 408 2.5 mg will begin receiving RTA 408 5 mg on Day 15 and the patients receiving placebo will remain on placebo in a blinded fashion. If an investigator cannot come to a firm decision whether or not to dose escalate a patient, he or she should discuss the relevant safety information with the medical monitor. Patients who are deemed inappropriate for dose escalation will continue on their originally randomized dose (i.e., 2.5 mg or placebo) throughout the remainder of the study. After the last patient in the initial cohort completes their Week 4 visit, the DSMB and Sponsor will review all available safety information from this cohort and make a recommendation regarding opening the next cohort to be randomized 3:1 to the cohort specific dose level of RTA 408 or placebo. All subsequent cohorts in Part 1, beginning with the second 8-patient cohort, will be evaluated for safety of the cohort specific dose-level by the DSMB once the eighth patient enrolled completes their Week 2 visit. The DSMB will recommend the dose of RTA 408 for the subsequent cohort. The DSMB dose recommendation for each cohort must not exceed 100% more than the highest dose of RTA 408 evaluated in this study, and the maximum permitted dose of RTA 408 is 160 mg. The dose-level for each new cohort will not exceed the DSMB recommended dose level, and it will be selected by the Sponsor based on review of available safety, efficacy, PK, and PD data.

7.4.3. Criteria for Determining Dose-Limiting Toxicity

The assessment of a DLT (i.e., a side effect serious enough to prevent an increase in dose) will be based on the clinical judgment of the investigator. Suspected DLTs should be discussed with the medical monitor. If an investigator cannot come to a firm decision whether or not to dose escalate a patient, he or she should discuss the relevant safety information with the medical monitor.

7.4.4. Intra-patient Dose Escalation

Intra-patient dose escalation will only occur for the first cohort of 8 patients randomized in Part 1 of the study (i.e., those patients randomized to RTA 408 2.5 mg or placebo). Investigators, and not the DSMB, will make intra-patient dose-escalation decisions during the first cohort of Part 1. Each patient will dose escalate starting on Day 15, unless a DLT is indicated by the investigator. Patients originally randomized to RTA 408 2.5 mg will dose escalate to RTA 408 5 mg, and those randomized to placebo will remain on placebo in a blinded fashion. If the investigator cannot come to a firm decision whether or not to dose escalate a patient, he or she should discuss the relevant safety information with the medical monitor. If the investigator determines the patient should not be dose escalated, that patient should continue on their originally randomized dose (i.e., 2.5 mg or placebo) throughout the remainder of the study visits.

No other patients are eligible for intra-patient dose escalation.

7.5. Data Safety Monitoring Board

A DSMB will review unblinded safety data throughout the study (i.e., both Part 1 and Part 2) and make recommendations as appropriate. The DSMB will begin monthly data reviews approximately 1 month after the first patient is enrolled in Part 1 through the last dose of the last patient enrolled in Part 2.

The DSMB will consist of external clinical experts supported by an independent statistical group. The independent statistical group will prepare unblinded analyses for the DSMB and will not have a role in the statistical analysis plan (SAP) after Part 2 of the study has started enrolling patients. A separate, blinded statistical group will be responsible for producing and finalizing the SAP and executing final data analysis of the study.

The DSMB will be governed by a charter that will describe the following:

- Roles and responsibilities of the DSMB members and the independent statistical group
- Meeting format and frequency
- Communication channels between the DSMB, the independent statistical group, the Sponsor, and the blinded study statisticians
- Voting process and requirements (e.g., requirement of consensus for issuance of a termination recommendation)
- Provisions governing conflict of interest and confidentiality

Briefly, the DSMB will review the progress of the study and the accumulating unblinded data while the study is ongoing. The DSMB will make recommendations to Sponsor representatives following each meeting. The DSMB may recommend that the study continue as is, be modified to

protect patient safety, or be terminated. In addition to the monthly reviews of unblinded study data, the DSMB will participate with the Sponsor in the recommendation to open the 10-mg cohort and additional dosing cohorts for enrollment for Part 1 and opening Part 2 described in Section 7.4.2. However, investigators, and not the DSMB, will make intra-patient dose-escalation decisions during the first cohort of Part 1.

7.6. Criteria for Study Termination

Although the Sponsor intends to complete the study, the Sponsor reserves the right to discontinue the study at any time for clinical or administrative reasons, or if required by regulatory agencies. If the Sponsor discontinues the study, all study treatment will be discontinued and the investigator will have the responsibility to prescribe any additional therapy to be administered.

7.7. Schedule of Assessments for Both Part 1 and Part 2

Table 3 lists the overall schedule of assessments for the study. Schedules of assessments for Part 1 and Part 2 are listed individually in Appendix 1. The order of assessments and duration of rest between assessments should be kept consistent within a patient across visits, to the extent possible.

Table 3: Overall Schedule of Assessments

Visit Number	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
Study Day/Week	Screening	Day 1	Week 1	Week 2	Week 4		Week 6	Week 8	Week 10	Week 12[a] End of treatment		Week 16 End of study/ 4-week follow-up
Day Relative to First Dose	-60 to -1[b]	1[c]	7[d] (±1 days)	14[e] (±3 days)	27 (±3 days)	28 (18-48 hr)[f]	42 (±3 days)	56 (±3 days)	70 (±3 days)	83 (±3 days)	84 (18-48 hr)[f]	112 (±3 days)
Informed consent	X											
Inclusion/Exclusion criteria assessment	X	X										
Demographics and baseline disease characteristics	X											
Prior and concomitant medication assessment	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X	X										
Height	X											
Echocardiogram	X[g]									X		
Electrocardiogram	X[g]	X		X[h]		X		X			X	X
Vital sign measurements	X	X	X	X	X	X		X		X	X	X
Weight and BMI	X	X	X	X	X	X		X		X	X	X
Physical examination	X									X		X
Adverse event collection		X	X	X	X	X	X	X	X	X	X	X
Clinical chemistry	X[i]	X	X	X		X		X			X	X
Hematology	X[i]	X	X	X		X		X			X	X
Urinalysis and microscopy	X[i]	X	X	X		X		X			X	X
BNP and NT-proBNP[j]	X	X	X	X		X		X			X	X
Hepatitis B and C and HIV[k]	X[i]											
Pregnancy test WOCBP[l]	X[i]	X		X		X		X			X	X
Randomization		X										
Study drug dispensation		X		X[m]		X		X				
Study drug return and pill count / diary[n]				X[o]		X		X			X	
Study drug administration[p]		<----------					X				---------->	
Telephone contact							X		X			
Exercise regimen reporting	X	X			X					X		
Maximal exercise test[q]	X				X					X		
Submaximal exercise test[r]		X				X					X	
6-minute walk test	X	X				X		X			X	
Fatigue Severity Scale		X								X		
SF-36 Health Survey Update		X								X		
Muscle needle biopsy[s]		X									X	
PK analysis				X[t,u]		X[v]		X[t]			X[t,u,v]	
Blood biomarkers[w]		X[w]								X		

[a] These procedures should be performed for early termination.

[b] Screening procedures for each patient should occur over the course of a few days and must finish at least 1 day prior to randomization. Study Day -1 is the day prior to first dose of study drug.

[c] All Day 1 procedures should be performed prior to administration of first dose of study drug.

[d] Assessments for Visit 3 may be collected at a home health nurse visit (at appropriate locations and approved by Sponsor) or at the study center clinic.

[e] Assessments for Visit 4 must be collected at the study center if patients are enrolled in Part 1 of the study. If patients are enrolled in Part 2 of the study, Visit 4 assessments may be collected by a home health nurse.

[f] Visit 6 should be conducted within 18 hours to 48 hours of Visit 5, and Visit 11 should be conducted within 18 hours to 48 hours of Visit 10.

[g] For patients with echocardiograms and electrocardiograms collected within 30 days prior to the Screening Visit, the most recent echocardiogram and electrocardiogram can be used to assess cardiac function and patient eligibility.

[h] At Week 2, electrocardiogram is only required for patients in Part 1 of the study.

[i] A home health nurse visit may be used to collect all lab samples required at the Screening Visit.

[j] Patients must be allowed to rest for a minimum period of 1 hour following maximal or submaximal exercise testing before this blood sample is collected (at the same time as all of the central lab blood collection). This sample must be taken prior to the 6MWT. This sample should be taken with the patient in the same position (e.g., sitting or semi-recumbent) at all appropriate visits

[k] Blood samples will be collected to analyze for hepatitis B and C and HIV antibodies only in patients lacking evidence of a negative titer in the past year.

[l] Negative serum pregnancy test results are required at the Screening Visit before study enrollment, and negative urine pregnancy test results are required at all other times indicated for continued participation in the study.

[m] At Week 2, study drug dispensation is only required for patients in the first cohort of Part 1 of the study.

[n] A dosing diary check must be performed at study drug return and pill count.

[o] At Week 2, study drug return, pill count, and diary check are only required for patients in the first cohort of Part 1 of the study.

[p] Study drug should be administered in the presence of study staff in the clinic on Day 1 after all Day 1 assessments have been completed. Study drug should also be administered in the clinic on Days 14, 28 (Part 2 only), 56 (Cohort 1; Part 1 only) and 84 after the blood collection for predose PK analysis. All other doses can be administered at home. RTA 408 should be administered once daily through Day 84.

[q] On study days where multiple assessments are to be completed, the maximal exercise test will be the first functional assessment performed. Blood samples will be collected at rest, at the completion of the test, and at 5 and 10 minutes after the test. Maximal exercise testing should be repeated prior to randomization if the original Screening assessment was performed >30 days prior to Day 1.

[r] As part of the submaximal exercise test, blood samples will be collected at rest and at 10-minute intervals throughout the test.

[s] Muscle needle biopsy, which is optional, should be collected after all study visit assessments are performed, except on Day 1, in which muscle needle biopsy should be the second to last procedure performed, collected prior to study drug administration.

[t] For patients enrolled in the first cohort of Part 1 of the study, blood samples for PK analysis at Visit 4 and Visit 8 should be collected prior to study drug administration as well as 1, 2, 4, and 8 hours after study drug administration. A single blood sample for PK analysis at Visit 11 should be collected prior to study drug administration.

[u] For patients enrolled in the second and subsequent cohorts of Part 1 of the study, blood samples for PK analysis at Visit 4 should be collected prior to study drug administration as well as 1, 2, 4, and 8 hours after study drug administration. A single blood sample for PK analysis at Visit 11 should be collected prior to study drug administration.

[v] For patients enrolled in Part 2 of the study, blood samples for PK analysis at Visit 6 and Visit 11 should be collected prior to study drug administration.

[w] Blood collection for biomarkers will be optional. On Day 1, blood collection for blood biomarkers must occur prior to study drug administration.

Abbreviations: 6MWT=6-minute walk test; BMI=body mass index; BNP= B-type natriuretic peptide; HIV=human immunodeficiency virus; NT-proBNP=N-terminal prohormone of B-type natriuretic peptide; PK=pharmacokinetic; WOCBP=women of childbearing potential

8. SELECTION AND WITHDRAWAL OF PATIENTS

8.1. Patient Inclusion Criteria

Patients must:

1. Have mitochondrial myopathy as evidenced by the following 2 criteria (must meet both):
    a. Have a history of exercise intolerance with or without weakness and/or progressive exercise intolerance (in which modest exercise typically provokes heaviness, weakness, aching of active muscles, or tachycardia)
    b. Have a known primary mitochondrial DNA mutation or a nuclear DNA defect that is associated with reduced activity of at least 1 mitochondrially encoded respiratory chain complex
2. Be male or female and ≥18 years of age and ≤75 years of age
3. Have no changes to their exercise regimen within 30 days prior to Study Day 1 and be willing to remain on the same exercise regimen during the 16-week study period
4. Have the ability to complete maximal exercise testing
5. Have peak work during maximal exercise testing of ≤1.5 W/kg
6. Have adequate kidney function defined as an estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease (MDRD) 4-variable formula
7. Be able to swallow capsules
8. Be willing and able to cooperate with all aspects of the protocol
9. Be willing to practice the medically acceptable methods of birth control (Section 9.7.2)
10. Provide written informed consent for study participation, approved by the appropriate Institutional Review Board (IRB) or Ethics Committee (EC)

8.2. Patient Exclusion Criteria

Patients must not:

1. Have uncontrolled diabetes (HbA1c >11.0%)
2. Have B-type natriuretic peptide (BNP) level >200 pg/mL
3. Have a history of clinically significant left-sided heart disease and/or clinically significant cardiac disease, including but not limited to any of the following:
    a. Clinically significant congenital or acquired valvular disease
    b. Pericardial constriction (based on echocardiogram performed at Screening Visit or within 30 days prior to Screening Visit)

   c. Restrictive or congestive cardiomyopathy (based on echocardiogram performed at Screening Visit or within 30 days prior to Screening Visit)
   d. Symptomatic coronary disease (prior myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft surgery, or angina)
   e. Evidence of left ventricular diastolic dysfunction
   f. History of atrial fibrillation
   g. History of unstable arrhythmias
   h. Cardiac insufficiency, defined as New York Heart Association Class >2
4. Have known active fungal, bacterial, and/or viral infection, including human immunodeficiency virus (HIV) or hepatitis virus (B or C)
5. Have known or suspected active drug or alcohol abuse, as per investigator judgment
6. Have clinically significant abnormalities of clinical hematology or biochemistry, including but not limited to elevations greater than 1.5 times the upper limit of normal of AST, ALT, or creatinine. Levels above this threshold are allowable if attributable to muscle injury
7. Have any abnormal laboratory test value or clinically significant pre-existing medical condition that, in the opinion of the investigator, would put the patient at risk by study enrollment
8. Have taken any of the following drugs within 7 days prior to Study Day 1 or plan to take any of these drugs during the time of study participation:
   a. Sensitive substrates for cytochrome P450 2C8 or 3A4 (e.g., repaglinide, midazolam, sildenafil)
   b. Moderate or strong inhibitors or inducers of cytochrome P450 3A4 (e.g., carbamazepine, phenytoin, ciprofloxacin, grapefruit juice)
   c. Substrates for p-glycoprotein transporter (e.g., ambrisentan, digoxin)
9. Have a history of clinically significant liver disease (e.g., fibrosis, cirrhosis, hepatitis), or has, at screening, clinically relevant deviations in laboratory tests including any one of the following:
   a. alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) > 1.5-fold ULN,
   b. bilirubin > 1.2-fold ULN,
   c. alkaline phosphatase (ALP) > 2-fold ULN,
   d. kidney insufficiency as defined by creatinine level > 1.5 mg/dL,
   e. albumin < lower limit of normal (LLN)
10. Have participated in any other interventional clinical study within 30 days prior to Study Day 1
11. Have a cognitive impairment that may preclude ability to comply with study procedures

12. Be unable to comply with the requirements of the study protocol or be unsuitable for the study for any reason, in the opinion of the investigator
13. Have used antioxidant supplements, including but not limited to idebenone, coenzyme Q10, nicotinamide, and vitamin E above the recommended daily allowance, within 14 days prior to Study Day 1 or plan to take any of these supplements during the time of study participation
14. Have taken chronic treatment with systemic corticosteroids within 30 days prior to Study Day 1
15. Have had significant suicidal ideation within 1 month prior to Screening Visit as per investigator judgment or any history of suicide attempts
16. Be pregnant or breastfeeding

8.3. Patient Rescreening

Patients may repeat the screening procedures once to qualify for the study if they have:

- Taken systemic corticosteroids within 30 days prior to Study Day 1 (rescreening may be performed 30 days or more after discontinuation of corticosteroids)
- Used antioxidant supplements, including but not limited to idebenone, coenzyme Q10, nicotinamide, and vitamin E above the recommended daily allowance, within 14 days prior to Study Day 1 (rescreening may be performed 14 days or more after discontinuation of supplement)
- Participated in any other interventional clinical study within 30 days prior to Study Day 1 (rescreening may be performed 30 days or more after completion of the clinical study)
- An abnormal laboratory test value that may normalize, in the opinion of the investigator (rescreening may be performed once test value is within normal range)
- Modified their exercise regimen within 30 days prior to Study Day 1 (rescreening may be performed 30 days or more after modification of exercise regimen)
- Taken any of the following drugs within 7 days prior to Study Day 1 (rescreening may be performed 7 days or more after discontinuation of the drug):
    - Sensitive substrates for cytochrome P450 2C8 or 3A4 (e.g., repaglinide, midazolam, sildenafil)
    - Moderate or strong inhibitors or inducers of cytochrome P450 2C8 or 3A4 (e.g., carbamazepine, phenytoin, ciprofloxacin, grapefruit juice)
    - Substrates for p-glycoprotein transporter (e.g., ambrisentan, digoxin)
- Failed screening because of operational challenges (rescreening may be performed no fewer than 2 weeks from the date of screen failure)

8.4. Patient Discontinuation and Termination

Patients have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. Furthermore, the investigator may discontinue a patient from study drug or terminate the patient from the study. The reason for a patient's withdrawal or discontinuation from the study will be recorded in the electronic case report form (eCRF).

8.4.1. Patient Discontinuation Criteria

Discontinuation refers to a patient stopping administration of study drug. Reasons for study drug discontinuation may include the following:

- Occurrence of an AE or change in medical status that leads the investigator to be concerned about the patient's welfare
- Protocol violations
- Administrative reasons (e.g., inability to continue)
- Sponsor termination of the study
- Voluntary withdrawal
- Pregnancy during the study
- Investigator unblinding

Patients who are discontinued from study drug should still complete all study visits and undergo all scheduled study assessments, if possible.

8.4.2. Patient Termination Criteria

Termination refers to a patient stopping study drug and all study assessments and visits. Reasons for study termination include the following:

- Administrative reasons (e.g., inability to continue, lost to follow-up)
- Death
- Withdrawal of consent

Patients who terminate the study for any reason may not re-initiate study drug or study assessments at any time.

9. TREATMENT OF PATIENTS

9.1. Description of Study Drug

Depending on cohort dose level (or doses selected for Part 2), patients will ingest either 3 or 4 capsules of study drug (1 from each bottle included in study treatment kit) daily throughout the study; study drug is described in Section 10.1. Treatment kits containing up to 30 mg of RTA 408 will include three bottles, each containing 30 count of either active or placebo capsules. Treatment kits containing greater than 30 mg of RTA 408 will be comprised of four bottles, each containing 30 count of either active or placebo capsules. To maintain the study blind, treatment kits used in Part 2 will all include the same number of bottles. The Sponsor will provide sufficient quantities of study drug to allow for completion of the study.

9.2. Prior and Concomitant Medications

9.2.1. Excluded Medications

Patients who have taken any of the following drugs within 30 days prior to Study Day 1 will be excluded from the study:

- Any other investigational drug
- Systemic corticosteroids

Patients who have taken any of the following drugs within 14 days prior to Study Day 1 will be excluded from the study:

- Antioxidant supplements, including but not limited to idebenone, coenzyme Q10, nicotinamide, and dosages exceeding the recommended daily allowance of vitamin E

Patients who have taken any of the following drugs within 7 days prior to Study Day 1 will be excluded from the study:

- Herbal preparations, minerals, or over-the-counter medication, except as identified in Section 9.2.2
- Sensitive substrates for cytochrome P450 2C8 or 3A4 (e.g., repaglinide, midazolam, sildenafil)
- Moderate or strong inhibitors or inducers of cytochrome P450 3A4 (e.g., carbamazepine, phenytoin, ciprofloxacin, grapefruit juice)
- Substrates for p-glycoprotein transporter (e.g., ambrisentan, digoxin)

The following medications and medication classes are not permitted during this study, except as noted in Section 9.2.2:

- Any other investigational drug
- Systemic corticosteroids
- Antioxidant supplements, including but not limited to idebenone, coenzyme Q10, nicotinamide, and dosages exceeding the recommended daily allowance of vitamin E

- Herbal preparations, vitamins, minerals, or over-the-counter medication, except as identified in Section 9.2.2
- Sensitive substrates for cytochrome P450 2C8 or 3A4 (e.g., repaglinide, midazolam, sildenafil)
- Moderate or strong inhibitors or inducers of cytochrome P450 3A4 (e.g., carbamazepine, phenytoin, ciprofloxacin, grapefruit juice)
- Substrates for p-glycoprotein transporter (e.g., ambrisentan, digoxin)
- Any interventional therapy intended to treat the mitochondrial myopathy disease condition, with the exception of a constant exercise regimen

9.2.2. Permitted Medications

The following concomitant medications are permitted, as authorized by the treating physician:

- Antibiotics, except as noted above (Section 9.2.1)
- Daily multivitamin
- Pain medication, except as noted above (Section 9.2.1)
- Other medications intended to manage concurrent diseases, except as noted above (Section 9.2.1)
- Oral, implantable, or injectable contraceptives

Patients taking medication chronically should be maintained on those same doses and dose schedules throughout the study period, as medically feasible. Patients taking medications with intermittent or as-needed schedules should try to avoid taking the concomitant medication on days when PK samples will be collected (i.e., Visits 4, 6, 8, and 11), as medically feasible.

9.3. Compliance With Study Drug

The investigator or designee will only dispense study drug to patients randomized to study treatment in accordance with the protocol. The study drug must not be used for reasons other than that described in the protocol.

All patients will return all bottles and unused capsules at the Weeks 4, 8 and 12 visits and will be dispensed a new kit of study drug at the Weeks 4 and 8 visits. In addition, patients in the first cohort of Part 1 will return all unused capsules at the Week 2 visit and will be dispensed a new kit of study drug at that time. Compliance will be measured by counting capsules to determine the number of missed doses from one visit to the next. Patients will also be provided a dosing diary to document that capsules were taken as instructed as well as the date and time of dosing (Section 9.9.20). To be considered compliant with study drug, patients can miss no more than 25% of the total planned doses (i.e., 21 doses) during the study. Patients who exceed the number of allowed missed doses will be considered noncompliant with dosing. Patients will not be discontinued from the study drug nor terminated from the study for noncompliance, but protocol deviations should be recorded for dosing noncompliance.

9.4. Randomization

Patients enrolled in Part 1 cohorts will be randomized 3:1 to RTA 408 at the cohort specific dose level or placebo. Patients enrolled in Part 2 will be randomized 1:1:1 to RTA 408 low dose, high dose, or placebo. Randomization for both Part 1 and Part 2 will be generated using a centralized IWRS. Randomization for Part 2 will be stratified by peak work at baseline (>0.8 W/kg vs. ≤0.8 W/kg).

9.5. Blinding

To maintain the study blind, all study drug kits will be packaged with blinded labels. Investigators will distribute the blinded study drug kits by kit number to patients as assigned by the IWRS. All patients, investigators, site personnel, and laboratories with direct involvement in the conduct of the study or their designees will be blinded to treatment assignments and appropriate measures will be taken to ensure the blind is maintained to reduce potential bias. Some Sponsor personnel may have access to treatment assignments during dose escalation (Part 1), but during Part 2, all Sponsor personnel will be blinded to treatment assignment.

The only people with access to treatment assignments for Part 2 will be those individuals who maintain the IWRS, the DSMB, the unblinded statistical team providing data to and analyzing data for the DSMB, and safety personnel without direct involvement in the conduct of the study who are assigned to report unblinded data to regulatory authorities as required.

9.5.1. Patient Unblinding

Although there is no known antidote to RTA 408, under rare circumstances, unblinding may be considered medically necessary for safety reasons. Unless faced with a life-threatening medical situation, the investigator should contact the medical monitor to discuss if there is a medically compelling reason to unblind the patient's treatment assignment. After the discussion, the investigator may proceed to unblind the patient, as appropriate. If unblinding is required, the investigator will utilize the IWRS to perform the unblinding. If a study drug assignment is unblinded, a description of the event that required unblinding must be documented by the investigator in the patient's source documents. Patients must discontinue taking study drug if their treatment assignment has been unblinded to the investigator (or designee), but they should remain blinded and continue attending all study visits and should undergo all study assessments. Patient treatment assignments must not be unblinded in the case of an AE or SAE, except as described above.

9.5.2. Unblinding for Regulatory Submission

In situations where a regulatory body requires unblinding and reporting of a particular SAE, the appropriate bodies (e.g., ethics committees, IRBs) must be provided with unblinded information according to the applicable regulatory requirement. This information must not be conveyed to the investigator, site personnel, or patient; therefore, this type of unblinding does not necessitate that the patient discontinue taking study drug.

9.6. Unscheduled Visits

Unscheduled visits are allowed for the following reasons:

- Patient rescreening
- Management of an AE or SAE
- Performance of additional laboratory tests for clinically abnormal test values or to confirm a possible pregnancy
- If the investigator feels that it is clinically appropriate for patient safety

9.7. Pregnancy

9.7.1. Women of Childbearing Potential

Women of childbearing potential (WOCBP) are female patients who are not surgically sterile (no history of bilateral tubal ligation, hysterectomy, or bilateral salpingo-oophorectomy), do not have fallopian inserts with confirmed blockage, have not had reproductive potential terminated by radiation or chemotherapy, and are not postmenopausal for at least 1 year.

9.7.2. Methods of Birth Control

9.7.2.1. Methods of Birth Control for Patients Enrolled in the United States:

From the Screening Visit, while taking study drug, and until 3 months after taking the final dose of study drug, WOCBP must agree to practice one of the following methods of birth control:

- Use of a double-barrier contraception method, defined as male use of a condom and female use of a barrier method (e.g., contraceptive sponge, spermicidal jelly or cream with or without a diaphragm)
- Use of hormonal contraceptives (oral, parenteral, vaginal, or transdermal) for at least 3 months prior to study drug administration
- Use of an intrauterine device
- Complete abstinence from sexual intercourse
- Male sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate.) The vasectomized partner should be the sole partner for that subject.

From the Screening Visit, while taking study drug, and until 3 months after taking the final dose of study drug, males who have female partners of childbearing potential must agree to practice one of the following methods of birth control:

- Have had a vasectomy (at least 6 months earlier)
- Use of a double-barrier contraception method, defined as male use of a condom and female use of a barrier method (e.g., contraceptive sponge, spermicidal jelly or cream with or without a diaphragm)

- Partner use of hormonal contraceptives (oral, parenteral, vaginal, or transdermal) for at least 3 months prior to study drug administration
- Partner use of an intrauterine device
- Complete abstinence from sexual intercourse

Male patients must also agree to not donate sperm starting on the first day of treatment until approximately 3 months after last dose of study drug.

9.7.2.2. Methods of Birth Control for Patients Enrolled in the European Union:

From the Screening Visit, while taking study drug, and until 3 months after taking the final dose of study drug, WOCBP must agree to practice one of the following highly effective methods of birth control:

- Use of hormonal contraceptives (contraceptive pills, implants, transdermal patches, hormonal vaginal devices or injections with prolonged release) for at least 3 months prior to study drug administration
- Use of an intrauterine device
- Complete abstinence from sexual intercourse if part of the subject's preferred lifestyle
- Male sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). The vasectomized partner should be the sole partner for that subject.

From the Screening Visit, while taking study drug, and until 3 months after taking the final dose of study drug, males who have female partners of childbearing potential must agree to practice one of the following methods of birth control:

- Have had a vasectomy (at least 6 months earlier)
- Partner use of hormonal contraceptives (contraceptive pills, implants, transdermal patches, hormonal vaginal devices or injections with prolonged release) for at least 3 months prior to study drug administration
- Partner use of an intrauterine device
- Complete abstinence from sexual intercourse if part of the subject's preferred lifestyle.

Male patients must also agree to not donate sperm starting on the first day of treatment until approximately 3 months after last dose of study drug.

9.7.3. Suspected Pregnancy

During the study, all WOCBP must be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., late or missed menstrual period). Male patients must be instructed to contact the investigator if a sexual partner suspects she may be pregnant.

If a patient or investigator suspects that the patient may be pregnant, the study drug must be withheld until the results of a serum pregnancy test are available. If pregnancy is confirmed with a serum pregnancy test, the patient must discontinue taking study drug but continue to attend all

study visits and undergo all study assessments. The investigator must immediately report a pregnancy associated with study drug exposure and record the event.

Pregnancy is not considered an AE; however, the investigator must follow a pregnant patient or the pregnant female partner of a male patient (if consenting) and report follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants resulting from such pregnancies should be followed for a minimum of 8 weeks. The Sponsor or its designee may contact the investigator to request additional information throughout the course of the pregnancy.

The following pregnancy outcomes must be considered SAEs and will require additional reporting in the case report form (CRF) and on an SAE form:

- Congenital anomaly/birth defect
- Stillbirth
- Spontaneous miscarriage

9.8. Treatment Interruption

In the case of serious toxicities, the investigator may choose to interrupt treatment with study drug. If study drug treatment interruption exceeds 21 total days, a protocol deviation should be noted. Dose reductions are not permitted. Patients who are permanently discontinued from study drug for any reason should still complete all study visits and undergo all scheduled study assessments, if possible. If the toxicity has resolved such that the investigator does not consider continued drug treatment to be an additional risk to the patient, the investigator may restart study drug treatment.

9.9. Study Procedures

To avoid inter-observer variability, every effort should be made to ensure that the same individual who made the initial baseline determination completes all subsequent assessments. All assessments occur in the study center clinic, with the exception of those assessments for Visits 3 (all patients) and 4 (patients in Part 2 only), which may be performed by a home health visit nurse (at appropriate locations and approved by Sponsor), and those assessments for Visits 7 and 9, which will be performed via telephone contact.

9.9.1. Informed Consent

Written informed consent (Section 17.3) must be obtained from the patient before any study-related procedures are performed, and if there is a change in the study procedures that could affect the patient's willingness to participate.

9.9.2. Inclusion/Exclusion Criteria

Inclusion and exclusion criteria should be reviewed at the times indicated in Table 3. Patients must meet all of the inclusion criteria and none of the exclusion criteria for entry into the study.

9.9.3. Demographics and Baseline Disease Characteristics

Demographic data, including sex, age, race, and ethnicity, and baseline disease characteristics, including documentation of evidence of mitochondrial myopathy, will be collected at the times indicated in Table 3. Documentation of mitochondrial myopathy includes genetic profiling of mtDNA or nuclear DNA mutations that underlie each patient's mitochondrial dysfunction.

9.9.4. Prior and Concomitant Medications

Information on prior and concomitant medications will be collected at the times indicated in Table 3. The name, dose, and frequency of all medications that the patient is taking or has taken within 30 days prior to informed consent must be recorded during the study and until the final visit. All allowed and excluded medications should be recorded, including all prescription drugs, herbal products, vitamins, minerals, and over-the-counter medications. Trade or generic drug names should be used whenever possible.

9.9.5. Medical History

A complete medical history (e.g., per patient report) that includes all medical history within the past 5 years will be collected and recorded at the time indicated in Table 3.

9.9.6. Height

Height should be measured without footwear, head coverings, or prosthetics at the time indicated in Table 3.

9.9.7. Weight and Body Mass Index

Weight in kilograms should be measured at the times indicated in Table 3. Body mass index (BMI) will be calculated within the eCRF each time the weight is recorded. If patients are weighed at the Screening Visit with additional leg braces, walkers, or shoes, all subsequent weight assessments must be conducted with the same appendages.

9.9.8. Echocardiogram

An echocardiogram will be recorded at the times indicated in Table 3 after the patient has rested for approximately 10 minutes in a supine position. For patients with echocardiograms collected within 30 days prior to Screening Visit, the most recent echocardiogram can be used to assess cardiac function and patient eligibility. Patients are ineligible to participate in the study if they have pericardial constriction or restrictive or congestive cardiomyopathy.

9.9.9. Electrocardiogram

A 12-lead electrocardiogram (ECG) will be recorded at the times indicated in Table 3 after the patient has rested for approximately 10 minutes in a supine position. The heart rate from the ECG machine should not be used as part of the vital sign measurements. For patients with electrocardiograms collected within 30 days prior to Screening Visit, the most recent electrocardiogram can be used to assess cardiac function and patient eligibility.

9.9.10. Vital Sign Measurements

Vital sign measurements should be taken at the times indicated in Table 3 and include the patient's heart rate (beats/minute), blood pressure (mm Hg), and body temperature (°C or °F). Blood pressure should be taken after the patient has rested in a sitting position for approximately 5 minutes. The same arm (usually the non-dominant arm) and the appropriate size cuff should be used for each measurement.

9.9.11. Physical Examination

A comprehensive physical examination must be performed by a physician, physician assistant, or registered nurse practitioner at the times indicated in Table 3. If possible, the same individual should perform each physical examination on a patient during the study. The examination must include the following organ or body system assessments: general appearance, head, eyes, ears, nose, throat, neck, musculoskeletal, cardiovascular, lymphatic, respiratory, abdomen, dermatologic, extremities, and neurological. Assessments of any specific signs or symptoms reported by the patient must also be performed and documented along with any other findings of note. Each body system finding must be assessed for clinical significance. Clinically significant findings at the Screening Visit must be included in the patient's medical history. New clinically significant findings after first dose must be included as AEs. Subsequent assessments beyond screening should be characterized as new, worsening, improved, or unchanged relative to the previous assessment.

9.9.12. Adverse Event Collection

Patients should be observed for general appearance, presence of illness or injury, or signs indicative of a concurrent illness at the times indicated in Table 3. Patients should be instructed to volunteer any information regarding AEs at any time during the study. The study physician or the study staff should also query patients with an open question regarding any AEs they may be experiencing (e.g., "How do you feel?" or "How have you been feeling since your last visit?"). Any findings are to be documented.

9.9.13. Clinical Chemistry

Blood samples will be collected for clinical chemistry analyses at the times indicated in Table 3. Clinical chemistry analyses are listed in Table 4. For calculation of the eGFR, the 4-variable MDRD equation must be used. The equation is as follows:

$$eGFR = 175 \times \text{standardized serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 1.212 \text{ [if black]} \times 0.742 \text{ [if female]}$$

Table 4: Clinical Chemistry Assessments

Clinical Chemistry Assessments
Blood urea nitrogen (BUN)
Creatinine
Total bilirubin
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Alkaline phosphatase (ALP)
Ferritin
Sodium
Potassium
Calcium
Inorganic phosphorus
Magnesium
Chloride
Bicarbonate
Uric acid
Cholesterol
Total protein
Glucose
Triglycerides
Albumin
Creatine phosphokinase (CPK)
Lactate dehydrogenase (LDH)
High-density lipoprotein cholesterol (HDL-C)
Low-density lipoprotein cholesterol (LDL-C)
Very-low-density lipoprotein cholesterol (VLDL-C)
Gamma-glutamyl transpeptidase (GGT)
Estimated glomerular filtration rate (eGFR) using the MDRD-4 formula

9.9.14. Hematology

Blood samples will be collected for hematology assessments at the times indicated in Table 3. Hematology assessments are listed in Table 5. As noted in Table 5, HbA1c will only be collected at Screening and Week 12 visits.

Table 5: Hematology Assessments

Hematology Assessments
Hematocrit
Hemoglobin
HbA1C (Screening, Week 12)
Red blood cell (RBC) count
White blood cell (WBC) count
Neutrophils
Bands (if detected)
Lymphocytes
Monocytes
Basophils (if detected)
Eosinophils (if detected)
Absolute platelet count
Mean corpuscular hemoglobin (MCH)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin concentration (MCHC)
Reticulocyte count

9.9.15. Urinalysis and Microscopy

Urine samples will be collected for urinalysis and microscopy assessments at the times indicated in Table 3. Urinalysis and microscopy assessments are listed in Table 6.

Table 6: Urinalysis/Microscopy Assessments

Urinalysis/Microscopy Assessments
Specific gravity
Ketones
pH
Protein
Blood
Glucose
Urobilinogen
Bilirubin
Microscopic examination (if indicated based on laboratory procedures)

9.9.16. N-Terminal Prohormone of B-Type Natriuretic Peptide and B-Type Natriuretic Peptide

Blood samples will be collected for N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) at the times indicated in Table 3 (as the same time as the central lab blood collection). As NT-proBNP may be affected by recent exercise, patients must be allowed to rest for a minimum period of 1 hour following maximal or submaximal exercise testing prior to obtaining this blood sample. Similarly, this sample must be taken prior to the 6MWT. This sample should be taken with the patient in the same position (e.g., sitting or semi-recumbent) at all appropriate visits.

9.9.17. Hepatitis B and C and Human Immunodeficiency Virus

Blood samples will be collected for hepatitis B and C and HIV antibody assessments only in patients lacking evidence of a negative titer in the past year at the time indicated in Table 3.

9.9.18. Pregnancy Test

WOCBP (Section 9.7.3) will provide a blood or urine sample for a pregnancy test at the times indicated in Table 3. Negative serum test results are required at the Screening Visit before study enrollment, and negative urine test results are required at all other times indicated in Table 3 for continued participation in the study. Any patient who becomes pregnant during the study must discontinue taking study drug immediately. See Section 9.7 for a description of procedures to be followed in case of pregnancy.

9.9.19. Study Drug Dispensation

Enough study drug will be dispensed to the patient at the times indicated in Table 3 to last at least until the next study visit, along with instructions for use.

9.9.20. Study Drug Return and Pill Count

Study drug will be collected from the patient and capsules will be counted by the principal investigator or the study staff at the times indicated in Table 3 to determine treatment compliance. Criteria for noncompliance and instructions for recording compliance on the CRF are included in Section 9.3.

Patients will be provided a dosing diary to track all doses administered, including the date and time of each dose. The patient will bring the dosing diary to the clinic to be reviewed by the site staff for study drug accountability.

9.9.21. Study Drug Administration

As outlined in Section 9.1, study drug treatment kits will contain either three or four 30-count bottles of RTA 408 or placebo capsules. Patients should take 1 capsule from each bottle daily. Patients should self-administer study drug orally once daily in the morning on an empty stomach (approximately 1 hour before or 2 hours after eating). Study drug should be taken with water at the times indicated in Table 3, except on Days 1, 14, 28 (Part 2 only), 56 (Cohort 1; Part 1 only), and 84 when study staff will administer study drug at the clinic following all assessments on Day 1, and after the pre-dose blood sample for PK on Days 14, 28 (Part 2 only), 56 (Cohort 1; Part 1 only), and 84. The date and time of the most recent 2 doses of study medication prior to Days 14, 28 (Part 2 only), 56 (Cohort 1; Part 1 only), and 84 will be recorded for PK analysis. The date and time of the last study drug administration (Day 84) should be recorded.

A vomited dose should not be replaced. Missed doses may be taken in the afternoon or evening of the same day approximately 1 hour before or 2 hours after a meal. A double dose (e.g., missed dose from previous day and dose for current day) should not be taken.

9.9.22. Telephone Contact

Sites should complete a telephone visit contact with the patient to assess AEs and concomitant medications at the times indicated in Table 3. Sites should make at least 3 attempts to contact the patient within the visit window if contact attempts are unsuccessful.

9.9.23. Exercise Regimen Reporting

Exercise regimen will be recorded at baseline. Patients will be asked about changes to their exercise regimen at the times indicated in Table 3. Changes to the exercise regimen during the study will be recorded as protocol deviations.

9.9.24. Maximal Exercise Test

Cycle ergometry using a recumbent stationary bicycle will be used to conduct maximal exercise testing at the times indicated in Table 3. Procedures for maximal exercise testing will be included in a study manual. Cardiac output measurements will only be done at select sites during maximal exercise testing. Maximal exercise testing assessments include but are not limited to peak work, peak oxygen utilization, and cardiac output. As part of the maximal exercise test, additional blood samples to measure serum lactate will be collected at rest, at the completion of the test, and at 5 and 10-minutes following the test. At Sponsor-qualified sites, blood samples to measure serum pyruvate, potassium, and glucose will also be collected at the same time points noted above.

9.9.25. Submaximal Exercise Test

Cycle ergometry will be used to conduct submaximal exercise tests at the times indicated in Table 3. Patients will perform the submaximal exercise testing at the subset of sites selected by the Sponsor, and submaximal exercise testing assessments will be required for all patients enrolling at these selected sites. Specific instructions will be provided in a study manual. Submaximal exercise test assessment may include but are not limited to submaximal work, peak serum lactate and pyruvate (optional), peak heart rate, and peak rating of perceived exertion. As part of the submaximal exercise test, additional blood samples to measure serum lactate will be collected at rest and at 10-minute intervals throughout the test. At Sponsor-qualified sites, blood samples to measure serum pyruvate, potassium, and glucose will be collected at rest and at 10-minute intervals throughout the test.

9.9.26. Six-Minute Walk Test

Patients will be instructed to walk as far as they can along a marked path in 6 minutes at the times indicated in Table 3. Distance walked will be measured. If patients use a cane or walking assist device at Screening, the same walking assist device must be used for all 6MWT assessments.

The 6MWT is a non-encouraged test that measures the distance walked over 6 minutes. The 6MWT must be performed consistently across all patients and sites. Therefore, each test must be performed in strict accordance with the instructions and script provided in Appendix 2, which are based on American Thoracic Society guidelines (ATS Statement, 2002).

[redacted]

[redacted]

9.9.32. Home Health Visits

Patients at approved sites will have the option of having a home health visit at the Screening Visit and Visit 3. If patients are enrolled in Part 2 of the study, Visit 4 assessments may be collected by a home health nurse. If a screening home health visit occurs, it will be used to collect all required lab samples for screening to help determine eligibility before the patient comes to the study clinic to complete all remaining screening activity. Once the patient has been enrolled in the study, the patient has the option of having a home health visit at Visit 3 and Visit 4 (for patients enrolled in Part 2 only) to complete the required lab collection, to have the home health nurse collect vital signs and weight, and to review any updates to AE information and concomitant medications.

10. STUDY DRUG MATERIALS AND MANAGEMENT

10.1. Study Drug

Capsules containing either RTA 408, in the 2.5-mg and 10-mg or 50-mg strengths, or the corresponding placebos, as described in Table 7, will be used in this study.

Table 7: Study Drug Information

Description	RTA 408 Capsule, 2.5 mg, 10 mg, 50 mg	Placebo Capsules
Ingredients	RTA 408, silicified microcrystalline cellulose, pregelatinized starch, croscarmellose sodium, magnesium stearate, in white opaque, size #4 or #0 capsule shells consisting of hypromellose and titanium dioxide	Silicified microcrystalline cellulose, pregelatinized starch, magnesium stearate, in white opaque, size #4 or #0 capsule shells consisting of hypromellose and titanium dioxide
Manufacturer	[redacted]	[redacted]

10.2. Study Drug Packaging and Labeling

RTA 408 active and placebo capsules are contained in identical primary container closure systems (i.e., 75-cc high-density polyethylene [HDPE] bottles with a foil induction-seal liner and a child-resistant closure). Treatment kits containing either three or four bottles, depending on treatment assignment will be provided to patients for self-administration. Each bottle will contain 30 capsules and a desiccant insert that must not be ingested. The label on each bottle will include the following information:

- Protocol 408-C-1403
- Medication ID number
- Caution statement: New Drug – Limited by Federal Law to Investigational Use (United States [US]); For Clinical Trial Use Only (Non-US countries)
- Storage: Controlled room temperature, 20°C to 25°C (68°F to 77°F in the US), with excursions allowed between 15°C and 30°C (59°F to 86°F in the US)
- Sponsor name, address, and contact information
- Contents: One bottle containing 30 capsules of either RTA 408 or placebo. Bottle also contains one desiccant insert, which should not be ingested
- Directions for use: Take capsule as directed, orally, once daily in the morning on an empty stomach (1 hour before or 2 hours after eating) with water
- Keep out of sight and reach of children

- FOR ORAL USE ONLY
- Lot number (Non-US countries)
- Expiration date (Non-US countries)
- Study contact (Non-US countries)
- EudraCT number: 2014-003501-15 (European Union countries)

Additionally, expiry information will be provided on treatment kits intended for use in the EU.

10.3. Study Drug Storage

The stability of the drug product is being evaluated in ongoing studies.

Investigative sites must store the investigational product in a secure location under controlled room temperature conditions of 20°C to 25°C (68°F to 77°F), with excursions allowed between 15°C and 30°C (59°F to 86°F). Sites must maintain documentation of appropriate storage conditions. If a temperature within the storage location at the site is noted to be outside the excursion range for 24 hours or more or exceeds 40°C, the Sponsor must be notified.

10.4. Study Drug Administration

Please refer to Section 9.9.21 for details on study drug administration. Patients will receive enough capsules for continuous dosing once daily at least until the next study visit. Clear instructions will be provided to the patient regarding study drug administration for each study drug administration time point listed in Table 3.

10.5. Study Drug Accountability

The investigator or designee will maintain a record of all study drug received, dispensed, and returned to the Sponsor or its designee. No study drug shall be destroyed by the clinical site unless directed to do so by the Sponsor or its designee. Study drug bottles and any unused capsules should be returned by the patient to the study staff.

10.6. Study Drug Handling and Disposal

The Sponsor or its designee will direct the site regarding the final disposition of any remaining study drug.

11. EFFICACY ASSESSMENTS

11.1. Maximal Exercise Test

The following parameters will be collected during the maximal exercise test and analyzed from Baseline to Weeks 4 and 12: change in peak work, peak oxygen utilization, and cardiac output at selected sites.

11.2. Submaximal Exercise Test

The following parameters will be collected during the submaximal exercise test and analyzed from Baseline to Weeks 4 and 12: change in peak serum lactate, change in peak serum pyruvate, change in peak heart rate, and change in the rating of perceived exertion.

11.3. Six-Minute Walk Test

The change in 6MWT distance from Baseline to Weeks 4, 8, and 12 will be analyzed.

[redacted]

[redacted]

12. SAFETY ASSESSMENTS

12.1. Safety Parameters

Safety parameters include results of echocardiogram, ECG, vital sign measurements, weight, BMI, physical examination, AEs, SAEs, concomitant medications, and laboratory tests (clinical chemistry, hematology, urinalysis, microscopy, and pregnancy tests [as indicated]).

12.2. Adverse and Serious Adverse Events

12.2.1. Definition of Adverse Events

12.2.1.1. Adverse Event

An AE is defined as any untoward medical occurrence in a patient regardless of its causal relationship to study treatment. An AE can be any unfavorable and unintended sign (including any clinically significant abnormal laboratory test result), symptom, or disease temporally associated with the use of the study drug, whether or not it is considered to be study drug related. Included in this definition is any newly occurring event or previous condition that has increased in severity or frequency since the administration of study drug.

All AEs that are observed or reported by the patient during the study (from the time of the first dose of study drug until the final visit or 30 days following final study dose for patients who terminate early) must be reported, regardless of their relationship to study drug or their clinical significance.

12.2.1.2. Serious Adverse Event

An SAE is any AE occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly or birth defect in an offspring of a patient taking study drug
- Is an important medical event

The term "life-threatening" refers to an event in which the patient was at immediate risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

Important medical events are those that may not meet any of the criteria defined above; however, they may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the SAE definition.

Pregnancy is not considered an AE; however, information will be collected for any pregnancies that occur during the study (from the time of first dose of study drug until the final visit). Certain pregnancy outcomes will require submission as an SAE (Section 9.7.3).

The investigator is responsible for reporting to the Sponsor or its designee all AEs and SAEs that are observed or reported by the patient during the study (from the time of first dose of study drug until the final visit), regardless of their relationship to study drug or their clinical significance. All SAEs reported or observed during the study must be followed to resolution or until the investigator deems the event to be chronic or the patient to be stable. The Sponsor or its designee may contact the investigator to obtain additional information on any SAE that has not resolved at the time the patient completes the study.

12.3. Eliciting Adverse Event Information

At every study visit, patients must be asked a standard, non-directed question, such as, "How do you feel?" or "How have you been feeling since your last visit?", to elicit any medically related changes in their well-being. They will also be asked if they have been hospitalized, had any accidents, used any new medications, or changed concomitant medication regimens (including prescription drugs, over-the-counter medications, vitamins, herbal products, and minerals). Responses should be recorded in the source documents.

In addition to patient observations, AEs must be documented for any clinically significant diagnosis resulting from abnormal laboratory test values, physical examination findings, ECG abnormalities, or other documents that are relevant to patient safety.

12.4. Assessment of Causality

The investigator must use the following classifications and criteria to characterize the relationship or association of the study drug in causing or contributing to the AE:

- Unrelated: This relationship suggests that there is no association between the study drug and the reported event.
- Unlikely: This relationship suggests that the temporal sequence of the event with study drug administration makes a causal relationship improbable and/or other factors also provide plausible explanations.
- Possible: This relationship suggests that treatment with the study drug caused or contributed to the AE. That is, the event follows a reasonable temporal sequence from the time of study drug administration and/or follows a known response pattern to the study drug but could have been produced by other factors.
- Probable: This relationship suggests that a reasonable temporal sequence of the event with study drug administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with study drug administration seems likely.

12.5. Assessment of Severity

The investigator will grade the severity of the AEs as mild, moderate, or severe using the following definitions:

- Mild: Symptoms causing no or minimal interference with usual social and functional activities
- Moderate: Symptoms causing greater than minimal interference with usual social and functional activities
- Severe: Symptoms causing inability to perform usual social and functional activities

12.6. Recording Adverse Events

All conditions present prior to the first dose of study drug should be documented as medical history. All drug-related (characterized as possibly or probably related; Section 12.4) AEs and abnormal laboratory test results reported or observed during the study must be followed to resolution (either return to baseline or within normal limits). All other AEs or non–drug-related abnormal laboratory results will be followed through the final visit (i.e., end of study or early termination). Information to be collected includes type of event, date of onset, date of resolution, investigator-specified assessment of severity and relationship to study drug, seriousness, as well as any action taken.

While an AE is ongoing, changes in the severity (e.g., worsening or improving) should be noted in the source documents but when documenting the AE, only the total duration and greatest severity should be recorded in the CRF. AEs characterized as intermittent require documentation of onset and duration.

AEs resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. Pre-existing conditions (present before the start of the AE collection period) are considered concurrent medical conditions and should NOT be recorded as AEs. However, if the patient experiences a worsening or complication of such a concurrent condition, the worsening or complication (except disease progression) should be recorded as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g., “worsening of…”).

Each AE should be recorded to represent a single diagnosis. Accompanying signs (including abnormal laboratory test values or ECG findings) or symptoms should NOT be recorded as additional AEs. If a diagnosis is unknown, sign(s) or symptom(s) should be recorded as an AE(s). Changes in laboratory test values or ECG parameters are only considered to be AEs if they are judged to be clinically significant (i.e., if some action or intervention is required or if the investigator judges the change to be beyond the range of normal physiological fluctuation). If abnormal laboratory test values or ECG findings are the result of pathology for which there is an overall diagnosis (e.g., increased creatinine levels in renal failure), only the diagnosis should be reported as an AE.

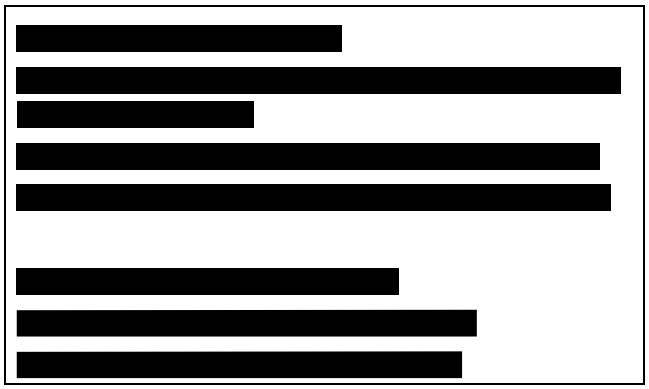
Elective procedures (surgeries or therapies) that were scheduled prior to the start of AE collection are not considered AEs. These elective procedures should not be recorded as AEs but should be documented in the patient’s source documents as elective (e.g., elective periodontal

surgery). However, if a preplanned procedure is performed early (e.g., as an emergency) because of a worsening of the pre-existing condition, the worsening of the condition should be captured as an AE.

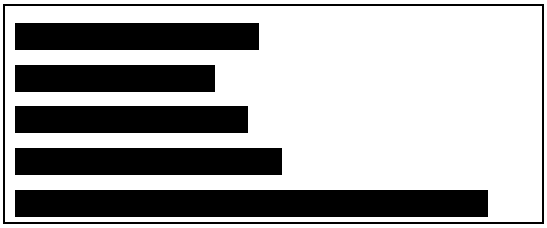
12.7. Reporting Serious Adverse Events

Any AE that meets the criteria of serious according to the previously described criteria must be reported within 24 hours from the time when site personnel first learn about the event. To report the SAE, fax the completed SAE form to Medpace (fax number listed below in Table 8) within 24 hours of awareness.

Table 8: Serious Adverse Event Reporting Contact Information



For questions regarding SAE reporting, contact your study manager, monitor, or medical monitor:



The investigator must continue to follow the patient until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the patient dies. Within 24 hours of receipt of new information, the updated follow-up SAE form, along with any supporting documentation (e.g., patient discharge summary or autopsy reports), should be faxed to Medpace Clinical Safety (Table 8).

The Sponsor or its designee will notify regulatory agencies of any fatal or life-threatening unexpected events associated with the use of the study drug as soon as possible but no later than 7 calendar days after the initial receipt of the information. Initial notification will be followed by a written report within the timeframe established by the appropriate regulatory agency. For other SAEs that do not meet the fatal or life-threatening unexpected criteria but are reported to be associated with the use of the study drug (that is, "possible" or "probable" in causality assessment), the Sponsor or its designee will notify the appropriate regulatory agencies in writing within the timeframe established by those regulatory agencies. The Sponsor or its designee will provide copies of any reports to regulatory agencies regarding serious and unexpected SAEs to the investigators for their information and submission to their IRB/EC, as appropriate.

Principal investigators are responsible for informing their IRB/EC of any SAEs at their site, as appropriate. The DSMB will review all safety data in an unblinded manner throughout the study and make recommendations as appropriate. SAE correspondence with regulatory authorities or IRBs/ECs must be submitted to the Sponsor or its designee for recording in the study file.

[redacted]

14. STATISTICS

14.1. Sample Size

The sample size for Part 1 is based on a dose-escalation scheme to evaluate initial safety, PK, and PD activity of RTA 408 in this patient population. The small number of patients at each dose in Part 1 is not expected to fully characterize safety, efficacy, or PD, but rather inform the DSMB and Sponsor of the appropriate doses to select for Part 2. With 24 patients enrolled in Part 2 (8 patients per treatment cohort), this study has approximately 80% power to detect an improvement of 0.28 W/kg in the peak work of pooled RTA 408 (n=16) versus placebo (n=8) assuming a two-sided Type I error rate of 0.05, a common within-group standard deviation (SD) of 0.28 W/kg, and a drop-out rate of no more than 1 in the placebo group and no more than 2 in the pooled RTA 408 group. The analysis will use repeated measures methodology, assuming compound symmetry for the within-subject covariance structure (ρ=0.20).

14.1.1. Sample Size Recalculation

A sample size recalculation will be performed in Part 2 after all 24 patients have been randomized to evaluate the pooled distribution of baseline peak work. If the SD for pooled, baseline peak work is greater than 0.28 W/kg or the distribution is not approximately normal, then an additional 12 patients will be enrolled in Part 2. With 36 patients enrolled in Part 2 (i.e., 12 patients per randomized treatment cohort), the 80% power is retained to detect the same difference of 0.28 W/kg if the SD is up to 0.35 W/kg, under the same assumptions of a two-sided Type I error rate of 0.05, and a drop-out rate of no more than 1 in the placebo group and no more than 2 in the pooled RTA 408 group, assuming compound symmetry for the within-subject covariance structure (ρ=0.20).

14.2. Study Variables

[redacted]

14.2.3. Efficacy Variables

Efficacy variables include parameters collected during maximal exercise testing (including peak work) and submaximal exercise testing; 6MWT distance; Fatigue Severity Scale results; and SF-36 scores.

14.2.4. Safety Variables

The safety variables include results of echocardiograms, ECGs, vital sign measurements, weight, BMI, physical examinations, AEs, SAEs, concomitant medications, and laboratory test results (clinical chemistry, hematology, urinalysis, microscopy, and pregnancy tests [as indicated]). All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and analyses of the AE data will be detailed in the SAP.

14.3. Statistical Analyses

A SAP detailing the analyses to be performed will be developed prior to the database lock. The SAP, which will describe in detail the methods used for the primary and secondary endpoints, will serve as the final arbiter of all statistical analyses.

Data will be summarized using descriptive statistics. Continuous data will be summarized with number of patients (n), mean, median, minimum, maximum, SD, coefficient of variation, and geometric mean (where applicable). Categorical data will be summarized using frequency counts and percentages.

14.3.1. Analysis Sets

The following analysis sets will be defined:

- The intent-to-treat (ITT) population includes all enrolled patients in both study parts (i.e., Part 1 and Part 2) categorized by their assigned treatment group (whether or not they received study drug).
- The modified ITT (mITT) population includes all patients enrolled in Part 2 of the study categorized by their assigned treatment group (whether or not they received study drug). Efficacy analyses will be performed using the mITT population.
- The safety population includes all patients who received at least 1 dose of study drug. Patients who receive at least 1 dose of RTA 408 will be classified in the RTA 408 group at the highest dose level received.
- Additional analysis sets may be defined in the SAP as appropriate.

14.3.2. Primary Efficacy Analysis

Primary analysis of the efficacy data will be based on the mITT population, which will include all patients randomized in Part 2 of the study. Peak work from Part 2 for the pooled patients treated with RTA 408 will be compared with placebo after 12 weeks of treatment by repeated measures analysis of covariance, with treatment group, time, and the interaction between treatment and time as fixed factors and baseline peak work as a covariate. The difference between RTA 408 and placebo in change from baseline of mean peak work will be estimated along with the 95% confidence interval of the difference. The SAP will describe the strategy used to control the Type I error rate when comparing each dose of RTA 408 with placebo$

15. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

15.1. Study Monitoring

The study monitor, as a representative of the Sponsor, has the obligation to follow the study conduct closely. In doing so, the monitor will visit the principal investigator and study facilities periodically, in addition to maintaining necessary telephone and letter contact. The monitor will maintain current knowledge of the study activity of the investigator and his/her staff through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigators and staff.

The Sponsor or its designee will monitor all aspects of the study for compliance with applicable government regulation with respect to the International Conference on Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice (GCP) E6(R1), abbreviated as ICH E6(R1), and current standard operating procedures.

Each principal investigator is expected to make a reasonable effort to accommodate the monitor when monitoring visits are necessary and to be available during the site visit. Furthermore, the monitor should be provided direct access to source data and documents for study-related monitoring and to the internet during the visit.

15.2. Audits and Inspections

Principal investigators and institutions involved in the study will permit study-related monitoring, audits, IRB/EC review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the principal investigator agrees to allow the Sponsor, representatives of the Sponsor, the US Food and Drug Administration (FDA), or other relevant regulatory authorities access to all study records.

The principal investigator or designee should promptly notify the Sponsor or designee of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the Sponsor or its designee.

16. QUALITY CONTROL AND QUALITY ASSURANCE

16.1. Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, the Sponsor may conduct a quality assurance audit. Please see Section 15.2 for more details regarding the audit process.

16.2. Financial Disclosure

Principal investigators and sub-investigators are required to provide financial disclosure information prior to starting the study. In addition, the principal investigator and sub-investigators must provide the Sponsor or its designee with updated information, if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Any potential investigator who has a vested financial interest in the success of this study may not participate in this study.

16.3. Sponsor Obligations

The Sponsor or its designee is not financially responsible for further testing/treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, the Sponsor or its designee is not financially responsible for treatment of non–study-related fatalities, physical injuries, or damage to health that may occur during the clinical study, as well as the patient's underlying disease.

16.4. Investigator Documentation

Before beginning the study, the principal investigator will be asked to comply with ICH E6(R1), Section 8.2 and Title 21 of the US Code of Federal Regulations (CFR), abbreviated as US CFR Title 21, by providing the essential documents to the Sponsor or its designee, which include but are not limited to the following:

- An original investigator-signed investigator agreement page of the protocol
- The IRB or EC approval of the protocol
- The IRB- or EC-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the patient or legal guardians
- A Form FDA 1572, fully executed, and all updates on a new fully executed Form FDA 1572
- Curriculum vitae for the principal investigator and each sub-investigator listed on Form FDA 1572. A curriculum vitae and current licensure, as applicable, must be provided. The curriculum vitae must have been signed and dated by the principal investigators and sub-investigators within 2 years before study start-up to indicate the documents are accurate and current

- Completed financial disclosure forms (Section 16.2) to allow the Sponsor or its designee to submit complete and accurate certification or disclosure statements required under US CFR Title 21, Part 54. In addition, the investigators must provide to the Sponsor or its designee a commitment to update this information promptly if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study
- Laboratory certifications and normal ranges for any laboratories used by the site for the conduct of this study

16.5. Clinical Study Insurance

In accordance with the respective national drug laws, the Sponsor has taken out patient liability insurance for all patients who give their consent and enroll in this study. This insurance covers potential study-related fatalities, physical injuries, or damage to health that may occur during the clinical study.

16.6. Use of Information

All information regarding RTA 408 supplied by the Sponsor to the investigator is privileged and confidential. The investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. Furthermore, the investigator is obligated to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of RTA 408 Capsules and may be disclosed to regulatory authority(ies), other investigators, corporate partners, or consultants, as required.

17. ETHICS

17.1. Institutional Review Board Review

The protocol and the proposed informed consent form (ICF) must be reviewed and approved by a properly constituted IRB/EC before study start. Each site must provide the Sponsor or its designee a signed and dated statement that the protocol and ICF have been approved by the IRB/EC before consenting patients. Prior to study initiation, the investigator is required to sign a protocol signature page confirming agreement to conduct the study in accordance with this protocol and to give access to all relevant data and records to the Sponsor, its designee, and regulatory authorities, as required.

The IRB/EC chairperson or designee must sign all IRB/EC approvals and must identify the IRB/EC by name and address, the clinical protocol, and the date approval and/or favorable opinion was granted.

The principal investigator is responsible for obtaining reviews of the clinical research at intervals specified by the IRB/EC, but not exceeding 1 year. The principal investigator must supply the Sponsor or its designee with written documentation of reviews of the clinical research.

17.2. Ethical Conduct of the Study

This clinical study was designed and shall be implemented and reported in accordance with the ICH E6(R1), with applicable local regulations (e.g., US CFR Title 21, European Directive 2001/20/EC), and with the ethical principles of the Declaration of Helsinki.

The principal investigator agrees to conduct the study in accordance with the ICH E6(R1) and the principles of the Declaration of Helsinki. The principal investigator must conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

17.3. Written Informed Consent

Because the study will be conducted under a US Investigational New Drug Application, a signed ICF, in compliance with US CFR Title 21, Part 50, will be obtained from each patient before the patient enters the study. An informed consent template may be provided by the Sponsor or its designee to the investigators. The consent must be reviewed by the Sponsor or its designee before IRB/EC submission. Once reviewed, the consent will be submitted by the principal investigator to his or her IRB/EC for review and approval before the start of the study. If the ICF is revised during the course of the study, all participants affected by the revision must sign the revised IRB- or EC-approved consent form in order to continue on the study.

Procedures that are to be performed as part of the practice of medicine and which would be done whether or not study entry was contemplated, such as for diagnosis or treatment of a disease or medical condition, may be performed and the results subsequently used for determining study eligibility without first obtaining consent (US FDA, 2014). Before enrollment, each prospective patient will be given a full explanation of the study and be allowed to read the approved ICF. Once the principal investigator or designee is assured that the patient understands the

implications of participating in the study, the patient will be asked to give consent to participate in the study by signing the ICF.

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), IRB- or EC-approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e., all of the procedures described in the protocol). The process of obtaining informed consent must be documented in the patient source documents.

Any changes to the proposed consent form suggested by the investigator must be agreed to by the Sponsor before submission to the IRB/EC, and a copy of the approved version and the notice of approval must be provided to the Sponsor's designated monitor after IRB or EC approval.

The principal investigator or designee will provide a copy of the ICF (signed copy to be provided per applicable law) to the patient and/or legal guardian. The original form will be maintained in the patient's medical records at the site.

17.4. Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain patient confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the patient (or the patient's guardian), except as necessary for monitoring and auditing by the Sponsor, its designee, the FDA or applicable regulatory authorities, or the IRB.

The principal investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished confidential information disclosed to them for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

17.5. Modification of the Protocol

Any changes that arise after the approval of the protocol must be documented as protocol amendments. FDA must be notified of protocol amendments. The changes will become effective only after approval of the Sponsor, the principal investigator, and the IRB. In cases where the protocol is modified to enhance patient safety, changes may be implemented and the amendment must be immediately submitted to the IRB.

The principal investigator is responsible for informing the IRB of all problems involving risks to patients according to national legislation. In case of urgent safety measures, the Sponsor will immediately notify FDA in accord with US CFR Title 21, Part 312, Section 32.

All substantial protocol amendments must also be submitted to the Danish Health and Medicines Authority (DHMA) and the Ethics Committee in Denmark and will become effective for sites in Denmark after approval of DHMA and the Ethics Committee.

17.6. Protocol Deviations

The principal investigator or designee must document any protocol deviations. The IRB/EC must be notified of all protocol deviations in a timely manner by the investigator as appropriate. Protocol deviations will be documented by the site personnel and reviewed by the responsible monitor during monitoring visits, and those observations will be communicated to the investigator.

If there is an immediate hazard to a patient, the principal investigator may deviate from the protocol without prior Sponsor and IRB/EC approval. The Sponsor and IRB/EC must be notified of the deviation.

18. DATA HANDLING AND RECORDKEEPING

18.1. Retention of Records

The investigator will maintain all study records according to ICH E6(R1) and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application is approved or 2 years after formal discontinuation of the clinical development of the investigational product. If the investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

18.2. Case Report Forms

All CRF data will be entered in electronic forms at the investigational site. The electronic data capture (EDC) system used to capture data electronically for all patients who signed informed consent will be US CFR Title 21, Part 11 compliant.

19. PUBLICATION POLICY

The Sponsor reserves the right to review all planned communications and manuscripts based on the results of this study. This reservation of the right is not intended to restrict or hinder publication or any other dissemination of study results, but to allow the Sponsor to confirm the accuracy of the data, to protect proprietary information, and to provide comments based on information that may not yet be available to the study investigators. The Sponsor supports communication and publication of study results whatever the findings of the study. The Sponsor also encourages disclosure of any conflict of interest from all authors or investigators when manuscripts are submitted for publication.

Those individuals who have contributed greatly to this study, as determined by the Sponsor, may serve on any publications committee for the study.

20. LIST OF REFERENCES

Adhihetty PJ, Taivassalo T, Haller RG, *et al*. The effect of training on the expression of mitochondrial biogenesis- and apoptosis-related proteins in skeletal muscle of patients with mtDNA defects. Am J Physiol Endocrinol Metab 2007;293:672-680.

[No authors listed]. ATS Statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-117.

Cejudo P, Bautista J, Montemayor T, *et al*. Exercise training in mitochondrial myopathy: a randomized controlled trial. Muscle Nerve 2005;32:342-350.

George L, Asghar M, Lokhandwala MF. Exercise stimulates transcription factors (Nrf2 & NFkB), increases antioxidant defenses, decreases oxidative stress, and restores renal dopamine D1 receptor function in aging. FASEB 2008;22:1159.6.

Goldstein A, Wolfe LA. The elusive magic pill: finding effective therapies for mitochondrial disorders. Neurotherapeutics 2013;10:320-328.

Holmström KM, Baird L, Zhang Y, *et al*. Nrf2 impacts cellular bioenergetics by controlling substrate availability for mitochondrial respiration. Biol Open 2013;2:761-770.

Honda T, Rounds BV, Bore L, *et al*. Novel synthetic oleanane triterpenoids: a series of highly active inhibitors of nitric oxide production in mouse macrophages. Bioorg Med Chem Lett 1999;9:3429-3434.

Jenkinson C, Stewart-Brown S, Petersen S, Paice C. Assessment of the SF-36 version 2 in the United Kingdom. J Epidemiol Community Health 1999;53:36-50.

Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The Fatigue Severity Scale: Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46:1121-1123.

Irving BA, Lanza I, Henderson G, *et al*. Effect of exercise training modality on skeletal muscle mitochondrial biogenesis in young and older adults. FASEB 2011;25:1107-1120.

Liu J. Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 1995;49:57-68.

Ludtmann MHR, Angelova PR, Zhang Y, *et al*. Nrf2 impacts the efficiency of mitochondrial fatty acid oxidation. Biochem J 2014;457:415-424.

Muthusamy VR, Kannan S, Sadhaasivam K, *et al*. Acute exercise stress activates Nrf2/ARE signaling and promotes antioxidant mechanisms in the myocardium. Free Rad Biol Med 2012;52:366-376.

Neymotin A, Calingasan NY, Wille E, *et al*. Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis. Free Rad Biol Med 2011;51:88-96.

Saha PK, Reddy VT, Konopleva M, et al. The triterpenoid 2-cyano-3,12-dioxooleana-1,9-dien-28-oic-acid methyl ester has potent anti-diabetic effects in diet-induced diabetic mice and Lepr(db/db) mice. J Biol Chem 2010;285:40581-40592.

Shin S, Wakabayashi J, Yates MS, Wakabayashi N, et al. Role of Nrf2 in prevention of high-fat diet-induced obesity by synthetic triterpenoid CDDO-imidazolide. Eur J Pharmcol 2009;620:138-144.

Taivassalo T, Shoubridge EA, Chen J, et al. Aerobic conditioning in patients with mitochondrial myopathies: physiological, biochemical, and genetic effects. Ann Neurol 2001;50;133-141.

Taivassalo T1, Gardner JL, Taylor RW, et al. Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions. Brain 2006;129(Pt 12):3391-3401.

Uruno A, Furusawa Y, Yagishita Y, et al. The Keap1-Nrf2 system prevents onset of diabetes mellitus. Mol Cell Biol 2013;33:2996-3010.

United States Food and Drug Administration [Web site]. Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation. Accessed July 21, 2014. Available at: http://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf

United States Food and Drug Administration [Web site]. Screening Tests Prior to Study Enrollment - Information Sheet. Guidance for Institutional Review Boards and Clinical Investigators. Accessed July 21, 2014. Available at: http://www.fda.gov/RegulatoryInformation/Guidances/ucm126430.htm

Wenz T, Diaz F, Spiegelman BM, et al. Activation of the PPAR/PGC-1α pathway prevents a bioenergetic deficit and effectively improves a mitochondrial myopathy phenotype. Cell Metab 2008;8:249-256.

21. APPENDICES

APPENDIX 1. SCHEDULE OF ASSESSMENTS FOR PART 1 AND PART 2

Table 9: Schedule of Assessments for Part 1

Visit Number	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
Study Day/Week	Screening	Day 1	Week 1	Week 2	Week 4		Week 6	Week 8	Week 10	Week 12[a] End of treatment		Week 16 End of study/ 4-week follow-up
Day Relative to First Dose	-60 to -1[b]	1[c]	7[d] (±1 days)	14[e] (±3 days)	27 (±3 days)	28 (18-48 hr)[f]	42 (±3 days)	56 (±3 days)	70 (±3 days)	83 (±3 days)	84 (18-48 hr)[f]	112 (±3 days)
Informed consent	X											
Inclusion/Exclusion criteria assessment	X	X										
Demographics and baseline disease characteristics	X											
Prior and concomitant medication assessment	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X											
Height	X											
Echocardiogram	X[g]									X		
Electrocardiogram	X[g]	X		X[h]		X		X			X	X
Vital sign measurements	X	X	X	X	X	X		X		X	X	X
Weight and BMI	X	X	X	X	X	X		X		X	X	X
Physical examination	X									X		X
Adverse event collection		X	X	X	X	X	X	X	X	X	X	X
Clinical chemistry	X[i]	X	X	X		X		X			X	X
Hematology	X[i]	X	X	X		X		X			X	X
Urinalysis and microscopy	X[i]	X	X	X		X		X			X	X
BNP and NT-proBNP[j]	X	X	X	X		X		X			X	X
Hepatitis B and C and HIV[k]	X[i]											
Pregnancy test WOCBP[l]	X[i]	X		X		X		X			X	X
Randomization		X										
Study drug dispensation		X		X[m]		X		X				
Study drug return and pill count / diary[n]				X[o]		X		X			X	
Study drug administration[p]		<----------	----------	----------	----------	----------	X	----------	----------	----------	----------	---------->
Telephone contact							X		X			
Exercise regimen reporting	X	X			X					X		
Maximal exercise test[q]	X				X					X		
Submaximal exercise test[r]		X				X					X	
6-minute walk test	X	X				X		X			X	
Fatigue Severity Scale		X								X		
SF-36 Health Survey Update		X								X		
Muscle needle biopsy[s]		X									X	
PK analysis				X[t,u]				X[t]			X[v]	
Blood biomarkers[w]		X[w]								X		

[a] These procedures should be performed for early termination.

[b] Screening procedures for each patient should occur over the course of a few days and must finish at least 1 day prior to randomization. Study Day -1 is the day prior to first dose of study drug.

[c] All Day 1 procedures should be performed prior to administration of first dose of study drug.

[d] Assessments for Visit 3 may be collected at a home health nurse visit (at appropriate locations and approved by Sponsor) or at the study center clinic.

[e] Assessments for Visit 4 must be collected at the study center if patients are enrolled in Part 1 of the study.

[f] Visit 6 should be conducted within 18 hours to 48 hours of Visit 5, and Visit 11 should be conducted within 18 hours to 48 hours of Visit 10.

[g] For patients with echocardiograms and electrocardiograms collected within 30 days prior to the Screening Visit, the most recent echocardiogram and electrocardiogram can be used to assess cardiac function and patient eligibility.

[h] At Week 2, electrocardiogram is only required for patients in Part 1 of the study.

[i] A home health nurse visit may be used to collect all lab samples required at the Screening Visit.

[j] Patients must be allowed to rest for a minimum period of 1 hour following maximal or submaximal exercise testing before this blood sample is collected (at the same time as all of the central lab blood collection). This sample must be taken prior to the 6MWT. This sample should be taken with the patient in the same position (e.g., sitting or semi-recumbent) at all appropriate visits

[k] Blood samples will be collected to analyze for hepatitis B and C and HIV antibodies only in patients lacking evidence of a negative titer in the past year.

[l] Negative serum pregnancy test results are required at the Screening Visit before study enrollment, and negative urine pregnancy test results are required at all other times indicated for continued participation in the study.

[m] At Week 2, study drug dispensation is only required for patients in the first cohort of Part 1 of the study.

[n] A dosing diary check must be performed at study drug return and pill count.

[o] At Week 2, study drug return, pill count, and diary check are only required for patients in the first cohort of Part 1 of the study.

[p] Study drug should be administered in the presence of study staff in the clinic on Day 1 after all Day 1 assessments have been completed. Study drug should also be administered in the clinic on Day 14, Day 56 (Cohort 1; Part 1 only) and Day 84 after the blood collection for predose PK analysis. All other doses can be administered at home. RTA 408 should be administered once daily through Day 84.

[q] On study days where multiple assessments are to be completed, the maximal exercise test will be the first functional assessment performed. Blood samples will be collected at rest, at the completion of the test, and at 5 and 10 minutes after the test. Maximal exercise testing should be repeated prior to randomization if the original Screening assessment was performed >30 days prior to Day 1.

[r] As part of the submaximal exercise test, blood samples will be collected at rest and at 10-minute intervals throughout the test.

[s] Muscle needle biopsy, which is optional, should be collected after all study visit assessments are performed, except on Day 1, in which muscle needle biopsy should be the second to last procedure performed, collected prior to study drug administration.

[t] For patients enrolled in cohort 1 of Part 1 of the study, blood samples for PK analysis at Visit 4 and Visit 8 should be collected prior to study drug administration as well as 1, 2, 4, and 8 hours after study drug administration.

[u] For patients enrolled in cohort 2 and subsequent cohorts of Part 1 of the study, blood samples for PK analysis at Visit 4 should be collected prior to study drug administration as well as 1, 2, 4, and 8 hours after study drug administration.

[v] Blood samples for PK analysis at Visit 11 should be collected prior to study drug administration

[w] Blood collection for biomarkers will be optional. On Day 1, blood collection for blood biomarkers must occur prior to study drug administration.

Abbreviations: 6MWT=6-minute walk test; BMI=body mass index; BNP= B-type natriuretic peptide; HIV=human immunodeficiency virus; NT-proBNP=N-terminal prohormone of B-type natriuretic peptide; PK=pharmacokinetic; WOCBP=women of childbearing potential

Table 10: Schedule of Assessments for Part 2

Visit Number	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
Study Day/Week	Screening	Day 1	Week 1	Week 2	Week 4		Week 6	Week 8	Week 10	Week 12[a] End of treatment		Week 16 End of study/ 4-week follow-up
Day Relative to First Dose	-60 to -1[b]	1[c]	7[d] (±1 days)	14[e] (±3 days)	27 (±3 days)	28 (18-48 hr)[f]	42 (±3 days)	56 (±3 days)	70 (±3 days)	83 (±3 days)	84 (18-48 hr)[f]	112 (±3 days)
Informed consent	X											
Inclusion/Exclusion criteria assessment	X	X										
Demographics and baseline disease characteristics	X											
Prior and concomitant medication assessment	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X	X										
Height	X											
Echocardiogram	X[g]									X		
Electrocardiogram	X[g]	X				X		X			X	X
Vital sign measurements	X	X	X	X	X	X		X		X	X	X
Weight and BMI	X	X	X	X	X	X		X		X	X	X
Physical examination	X									X		X
Adverse event collection		X	X	X	X	X	X	X	X	X	X	X
Clinical chemistry	X[h]	X	X	X		X		X			X	X
Hematology	X[h]	X	X	X		X		X			X	X
Urinalysis and microscopy	X[h]	X	X	X		X		X			X	X
BNP and NT-proBNP[i]	X	X	X	X		X		X			X	X
Hepatitis B and C and HIV[j]	X[h]											
Pregnancy test WOCBP[k]	X[h]	X		X		X		X			X	X
Randomization		X										
Study drug dispensation		X				X		X				
Study drug return and pill count / diary[l]						X		X			X	
Study drug administration[m]		<----------	----------	----------	----------	----------	X	----------	----------	----------	----------	---------->
Telephone contact							X		X			
Exercise regimen reporting	X	X			X					X		
Maximal exercise test[n]	X				X					X		
Submaximal exercise test[o]		X				X					X	
6-minute walk test	X	X				X		X			X	
Fatigue Severity Scale		X								X		
SF-36 Health Survey Update		X								X		
Muscle needle biopsy[p]		X									X	
PK analysis[q]						X					X	
Blood biomarkers[r]		X[r]								X		

[a] These procedures should be performed for early termination.

[b] Screening procedures for each patient should occur over the course of a few days and must finish at least 1 day prior to randomization. Study Day -1 is the day prior to first dose of study drug.

[c] All Day 1 procedures should be performed prior to administration of first dose of study drug.

[d] Assessments for Visit 3 may be collected at a home health nurse visit (at appropriate locations and approved by Sponsor) or at the study center clinic.

[e] If patients are enrolled in Part 2 of the study, Visit 4 assessments may be collected by a home health nurse.

[f] Visit 6 should be conducted within 18 hours to 48 hours of Visit 5, and Visit 11 should be conducted within 18 hours to 48 hours of Visit 10.

[g] For patients with echocardiograms and electrocardiograms collected within 30 days prior to the Screening Visit, the most recent echocardiogram and electrocardiogram can be used to assess cardiac function and patient eligibility.

[h] A home health nurse visit may be used to collect all lab samples required at the Screening Visit.

[i] Patients must be allowed to rest for a minimum period of 1 hour following maximal or submaximal exercise testing before this blood sample is collected (at the same time as all of the central lab blood collection). This sample must be taken prior to the 6MWT. This sample should be taken with the patient in the same position (e.g., sitting or semi-recumbent) at all appropriate visits

[j] Blood samples will be collected to analyze for hepatitis B and C and HIV antibodies only in patients lacking evidence of a negative titer in the past year.

[k] Negative serum pregnancy test results are required at the Screening Visit before study enrollment, and negative urine pregnancy test results are required at all other times indicated for continued participation in the study.

[l] A dosing diary check must be performed at study drug return and pill count.

[m] Study drug should be administered in the presence of study staff in the clinic on Day 1 after all Day 1 assessments have been completed. Study drug should also be administered in the clinic on Day 28 and Day 84 after the blood collection for predose PK analysis. All other doses can be administered at home. RTA 408 should be administered once daily through Day 84.

[n] On study days where multiple assessments are to be completed, the maximal exercise test will be the first functional assessment performed. Blood samples will be collected at rest, at the completion of the test, and at 5 and 10 minutes after the test. Maximal exercise testing should be repeated prior to randomization if the original Screening assessment was performed >30 days prior to Day 1.

[o] As part of the submaximal exercise test, blood samples will be collected at rest and at 10-minute intervals throughout the test.

[p] Muscle needle biopsy, which is optional, should be collected after all study visit assessments are performed, except on Day 1, in which muscle needle biopsy should be the second to last procedure performed, collected prior to study drug administration.

[q] Blood samples for PK analysis should be collected prior to study drug administration.

[r] Blood collection for biomarkers will be optional. On Day 1, blood collection for blood biomarkers must occur prior to study drug administration.

Abbreviations: 6MWT=6-minute walk test; BMI=body mass index; BNP= B-type natriuretic peptide; HIV=human immunodeficiency virus; NT-proBNP=N-terminal prohormone of B-type natriuretic peptide; PK=pharmacokinetic; WOCBP=women of childbearing potential

APPENDIX 2. SIX-MINUTE WALK TEST INSTRUCTIONS

(Modified from American Thoracic Society (ATS) Statement: Guidelines for the Six-Minute Walk Test. [ATS Statement, 2002])

PURPOSE AND SCOPE

This statement provides practical guidelines for the 6-minute walk test (6MWT). Specifically, it reviews indications, details factors that influence results, presents a brief step-by-step protocol, outlines safety measures, describes proper patient preparation and procedures, and offers guidelines for clinical interpretation of results.

STUDY-SPECIFIC REQUIREMENTS

The assessor of the 6MWT must not provide the patient with the results of the assessment once complete.

BACKGROUND

The 6MWT is a practical simple test that requires a 100-ft (30-meter) hallway but no exercise equipment or advanced training for technicians. Walking is an activity performed daily by all but the most severely impaired patients. This test measures the distance that a patient can walk on a flat, hard surface in a period of 6 minutes. It evaluates the global and integrated responses of all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism. It does not provide specific information on the function of each of the different organs and systems involved in exercise or the mechanism of exercise limitation, as is possible with maximal cardiopulmonary exercise testing. The self-paced 6MWT assesses the submaximal level of functional capacity. Most patients do not achieve maximal exercise capacity during the 6MWT; instead, they choose their own intensity of exercise and are allowed to stop and rest during the test. However, because most activities of daily living are performed at submaximal levels of exertion, the 6MWT may better reflect the functional exercise level for daily physical activities.

CONTRAINDICATIONS

Absolute contraindications for the 6MWT include the following: unstable angina during the previous month and myocardial infarction during the previous month. Relative contraindications include a resting heart rate of more than 120, a systolic blood pressure of more than 180 mm Hg, and a diastolic blood pressure of more than 100 mm Hg.

Patients with any of these findings should be referred to the physician ordering or supervising the test for individual clinical assessment and a decision about the conduct of the test. The results from a resting electrocardiogram (ECG) done during the previous 6 months should also be reviewed before testing. Stable exertional angina is not an absolute contraindication for a 6MWT, but patients with these symptoms should perform the test after using their antiangina medication, and rescue nitrate medication should be readily available.

RATIONALE

Patients with the previously mentioned risk factors may be at increased risk for arrhythmias or cardiovascular collapse during testing. However, each patient determines the intensity of their

exercise, and the test (without ECG monitoring) has been performed in thousands of older persons and thousands of patients with heart failure or cardiomyopathy without serious adverse events. The contraindications listed previously here were used by study investigators based on their impressions of the general safety of the 6MWT and their desire to be prudent, but it is unknown whether adverse events would occur if such patients performed a 6MWT; they are, therefore, listed as relative contraindications.

SAFETY ISSUES

1. Testing should be performed in a location where a rapid, appropriate response to an emergency is possible. The appropriate location of a crash cart should be determined by the physician supervising the facility.
2. Supplies that must be available include oxygen, sublingual nitroglycerine, aspirin, and albuterol (metered dose inhaler or nebulizer).
3. A telephone or other means should be in place to enable a call for help.
4. The technician should be certified in cardiopulmonary resuscitation with a minimum of Basic Life Support by an American Health Association–approved cardiopulmonary resuscitation course. Advanced cardiac life support certification is desirable. Training, experience, and certification in related health care fields (registered nurse, registered respiratory therapist, certified pulmonary function technician, etc.) are also desirable. A certified individual should be readily available to respond if needed.
5. Physicians are not required to be present during all tests. The physician ordering the test or a supervising laboratory physician may decide whether physician attendance at a specific test is required.
6. If a patient is on chronic oxygen therapy, oxygen should be given at their standard rate or as directed by a physician or a protocol.

Reasons for immediately stopping a 6MWT include the following: (1) chest pain, (2) intolerable dyspnea, (3) leg cramps, (4) staggering, (5) diaphoresis, and (6) pale or ashen appearance.

Technicians must be trained to recognize these problems and the appropriate responses. If a test is stopped for any of these reasons, the patient should sit or lie supine as appropriate depending on the severity or the event and the technician's assessment of the severity of the event and the risk of syncope. The following should be obtained based on the judgment of the technician: blood pressure, pulse rate, oxygen saturation, and a physician evaluation. Oxygen should be administered as appropriate.

TECHNICAL ASPECTS OF THE 6MWT

Location

The 6MWT should be performed indoors, along a long, flat, straight, enclosed corridor with a hard surface that is seldom traveled. The walking course must be 15-30 meters in length. An approximately 49- to 98-ft hallway is, therefore, required. The length of the corridor should be marked every 3 meters. The turnaround points should be marked with a cone (such as an orange traffic cone). A starting line, which marks the beginning and end of each lap, should be marked on the floor using brightly colored tape.

Rationale
A shorter corridor requires patients to take more time to reverse directions more often, reducing the 6MWT.

REQUIRED EQUIPMENT

1. Countdown timer (or stopwatch)
2. Mechanical lap counter
3. Two small cones to mark the turnaround points
4. A chair that can be easily moved along the walking course
5. Worksheets on a clipboard
6. A source of oxygen
7. Sphygmomanometer
8. Telephone
9. Automated electronic defibrillator

PATIENT PREPARATION

1. Comfortable clothing should be worn.
2. Appropriate shoes for walking should be worn. A notation should be made in the patient's source documentation of which shoes the patient wore for the first test. Prior to each clinic visit the patient should be called/reminded to ensure they wear this same pair of shoes.
3. Patients should use their usual walking aids during the test (cane, walker, etc.). NOTE – if a patient uses a walking aid for the first test, this same aid should be used during all subsequent test whether needed or not.
4. The patient's usual medical regimen should be continued. The patient should continue to take doses of study drug prior to the test.
5. A light meal is acceptable before early morning or early afternoon tests.
6. Patients should not have exercised vigorously within 2 hours of beginning the test.

MEASUREMENTS

1. Repeat testing should be performed about the same time of day to minimize intraday variability.
2. A "warm-up" period before the test should not be performed.
3. The patient should sit at rest in a chair, <u>located at the starting position</u>, for at least 10 minutes before the test starts. During this time, check for contraindications, measure pulse and blood pressure, and make sure that clothing and shoes are appropriate. Also, note the patient's general conditions and ailments (i.e., physical or mental) on this day that may potentially influence the results of the test. Complete the first portion of the course document worksheet.

4. Pulse oximetry is optional. If it is performed, measure and record baseline heart rate and pulse oximetry oxygen saturation (SpO_2) and follow manufacturer's instructions to maximize the signal and to minimize motion artifact. Make sure the readings are stable before recording. Note pulse regularity and whether the oximeter signal quality is acceptable.

The rationale for measuring oxygen saturation is that although the distance is the primary outcome measure, improvement during serial evaluations may be manifest either by an increased distance or by reduced symptoms with the same distance walked. The SpO_2 should not be used for constant monitoring during the exercise. The technician must not walk with the patient to observe the SpO_2. If worn during the walk, the pulse oximeter must be lightweight (less than 2 pounds), battery powered, and held in place (perhaps by a "fanny pack") so that the patient does not have to hold or stabilize it and so that stride is not affected. Many pulse oximeters have considerable motion artifact that prevents accurate readings during the walk.

1. Set the lap counter to zero and the timer to 6 minutes. Assemble all necessary equipment (lap counter, timer, clipboard, Borg Scale, worksheet) and move to the starting point.
2. Instruct the patient as follows:

"The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.

You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn without hesitation."

Demonstrate by walking one lap yourself. Walk and pivot **around** a cone briskly.

"Are you ready to do that? I am going to use this counter to keep track of the number of laps you complete. I will click it each time you turn around at this starting line. Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog. Start now, or whenever you are ready."

1. Position the patient at the starting line. You should also stand near the starting line during the test. Do not walk with the patient. As soon as the patient starts to walk, start the timer.
2. **Do not talk to anyone during the walk.** Use an **even tone of voice** when using the standard phrases of encouragement. Watch the patient. **Do not get distracted and lose count of the laps.** Each time the participant returns to the starting line, click the lap counter once (or mark the lap on the worksheet). Let the participant see you do it. Exaggerate the click using body language, like using a stopwatch at a race.

After the first minute, tell the patient the following (in even tones): *"You are doing well. You have 5 minutes to go."*

When the timer shows 4 minutes remaining, tell the patient the following: *"Keep up the good work. You have 4 minutes to go."*

When the timer shows 3 minutes remaining, tell the patient the following: *"You are doing well. You are halfway done."*

When the timer shows 2 minutes remaining, tell the patient the following: *"Keep up the good work. You have only 2 minutes left."*

When the timer shows only 1 minute remaining, tell the patient the following: *"You are doing well. You have only 1 minute to go."*

Do not use other words of encouragement (or body language to speed up).

If the patient stops walking during the test and needs a rest, say this: *"You can lean against the wall if you would like; then continue walking whenever you feel able."* **Do not stop the timer**. If the patient stops before the 6 minutes are up and refuses to continue (or you decide that they should not continue), wheel the chair over for the patient to sit on, discontinue the walk, and note on the worksheet the distance, the time stopped, and the reason for stopping prematurely.

When the timer is 15 seconds from completion, say this: *"In a moment I'm going to tell you to stop. When I do, just stop right where you are and I will come to you."*

When the timer rings (or buzzes), say this: *"Stop!"* Walk over to the patient. Consider taking the chair if they look exhausted. Mark the spot where they stopped by placing a bean bag or a piece of tape on the floor.

1. Post-test: Record the post-walk Borg dyspnea and fatigue levels and ask this: "What, if anything, kept you from walking farther?"
2. If using a pulse oximeter, measure SpO_2 and pulse rate from the oximeter and then remove the sensor.
3. Record the number of laps from the counter (or tick marks on the worksheet).
4. Record the additional distance covered (the number of meters in the final, partial lap) using the markers on the wall as distance guides.
5. Calculate the total distance walked, rounding to the nearest meter (i.e., 251.3 meters is 251 meters or 251.5 meters is 252 meters), and record it on the worksheet.
6. Congratulate the patient on good effort and offer a drink of water.

QUALITY ASSURANCE

Sources of Variability

There are many sources of 6MWT variability. The sources of variability caused by the test procedure itself should be controlled as much as possible. This is done by following the standards found in this document and by using a quality assurance program.

Technician Training and Experience

Technicians who perform 6MWT should be trained using the standard protocol and then supervised for several tests before performing them alone. They should also have completed cardiopulmonary resuscitation training.

Encouragement
Only standardized phrases for encouragement (as outlined above) must be used during the test.

Rationale
Encouragement significantly increases the distance walked. Reproducibility for tests with and without encouragement is similar. We have chosen every minute and standard phrases.

Supplemental Oxygen
If oxygen supplementation is utilized chronically, then it should be utilized during the walks and if serial tests are planned (after an intervention other than oxygen therapy), then during all walks by that patient oxygen should be delivered in the same way with the same flow. If the flow must be increased during subsequent visits due to worsening gas exchange, this should be noted on the worksheet and considered during interpretation of the change noted in 6MWT. The type of oxygen delivery device should also be noted on the report: for instance, the patient carried liquid oxygen or pushed or pulled an oxygen tank, the delivery was pulsed or continuous, or a technician walked behind the patient with the oxygen source (not recommended). Measurements of pulse and SpO_2 should be made after waiting at least 10 minutes after any change in oxygen delivery.

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