# A RANDOMIZED, DOUBLE-BLIND, CONTROLLED, PHASE 2/3 STUDY TO ASSESS EFFICACY, LONG TERM SAFETY AND TOLERABILITY OF RT001 IN SUBJECTS WITH FRIEDREICH'S ATAXIA

## PROTOCOL NUMBER: RT001-006

Version 8.0
Protocol Date: 03 Nov 2021

### Confidentiality Statement

# 1. SIGNATURE PAGE

Title of Protocol: A Randomized, Double-Blind, Controlled, Phase 2/3 Study to Assess Efficacy, Long Term Safety and Tolerability of RT001 in Subjects with Friedreich's Ataxia

Protocol No: RT001-006

## 1.1. Sponsor Approval

Peter L Milner — NOV 3, 2021

**Peter Milner, MD** — Date
Chief Medical Officer/Sponsor Representative

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## 1.2. Investigator Agreement

By signing this page, I attest that I have read and understand the contents of Clinical Protocol RT001-006. I agree to adhere to the design, conduct, and reporting requirements of the study as stated in the clinical protocol and to my obligations to the Sponsor as described in the protocol, The Declaration of Helsinki, relevant 21 CFR sections, Good Clinical Practices, and the executed contracts between myself, my Institution, and the Sponsor.

_____ _____

Signature **Investigator Signature** Date

Name and Address of Institution: _____

# 2. STUDY SYNOPSIS

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|---|---|
| **Sponsor:** | Retrotope, Inc.<br>4300 El Camino Real, Suite 201<br>Los Altos, CA 94022 |
| **Title:** | A Randomized, Double-Blind, Controlled, Phase 2/3 Study to Assess Efficacy, Long Term Safety and Tolerability of RT001 in Subjects with Friedreich's Ataxia |
| **Protocol No:** | RT001-006 |
| **Study Drugs:** | **Active**: RT001, 960 mg soft gelatin capsules (9-*cis*, 12-*cis*-11,11-$D_2$-linoleic acid ethyl ester [deuterated ethyl linoleate, or $D_2$-LA])<br>**Placebo**: Soft gelatin capsule of USP safflower oil (Placebo or $H_2$-LA) |
| **Study Population:** | Friedreich's ataxia (FRDA) patients who meet all entry criteria. |
| **Number of Subjects** | The planned enrollment is 60 subjects randomized 1:1 (active: placebo).<br>Randomization will be stratified by Friedreich Ataxia Rating Scale (FARS) Neurological score: ≤45 and >45<br>Enrollment of patients with age > 25 will be capped at 25 % of the total enrollment |
| **Dosing Schedule** | Subjects will receive the content of nine capsules daily (8.64 g total dose) given as 3 capsules three times a day (TID) with meals for the first month of treatment. After this initial loading period, the dose will be reduced to six capsules daily (5.76 g total dose) given as 3 capsules with breakfast, and 3 capsules with dinner for the remainder of the trial, while on study drug.<br>If a subject is unable to tolerate study drug because of adverse events during the initial loading period, the dose may be reduced to six capsules daily (5.76 g total dose) given as 3 capsules with breakfast, and 3 capsules with dinner.<br>If a subject is unable to tolerate study drug because of adverse events after the dose reduction to six capsules daily (5.76 g total dose), the dose may be spread over three meals (2 capsules at breakfast, 2 capsules with lunch, and 2 capsules with dinner). If the subject is still unable to tolerate study drug after spreading it over three meals, the total dose may be reduced by 1-2 capsules/day, preferably by reducing the dose for a meal from 2 to 1 capsule(s) as needed. After 2 weeks of taking a dose of less than 6 caps/day, the subject should try to increase the dose to 6 capsules/day given as 2 capsules TID given with meals. If 6 caps/day is not tolerable, the subject can take the drug at the dose of less than 6 caps/day that was tolerable. |
| **Route of Administration:** | Oral |
| **Study Centers:** | up to 10 study centers |
| **Phase of Development** | Phase 2/3 |

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| **Objectives** | **Primary endpoints:**<br>1. To demonstrate the efficacy of RT001 compared to placebo on the change of MVO2 in patients with FA through change from baseline to 11 months<br>**Key secondary endpoint:**<br>1. To demonstrate the efficacy of RT001 compared to placebo on an overall performance score based on a Global Statistical Test (GST) change from baseline to 11 months. The GST will be a combination of three endpoints (timed 1 minute walk test [T1MW], peak workload, and maximum rate of oxygen consumption [MVO2]).<br>**Secondary endpoints:**<br>1. To demonstrate the efficacy of RT001 compared to placebo on T1MW with change from baseline to 11 months<br>2. To demonstrate the efficacy of RT001 compared to placebo on the change of peak workload measured by CPET through change from baseline to 11 months.<br>**Exploratory endpoints:**<br>To demonstrate the efficacy of RT001 on change from baseline at 11 months in clinical symptoms as measured by:<br>1. Modified Friedreich's Ataxia Scale (mFARS)<br>2. Fatigue Symptom Scale (items 2. 4. 7.9 of the total fatigue scale)<br>3. Clinician-rated Clinical Global Impression of Change (CGI-C)<br>4. Activities of daily living (ADL) elements of fatigue scale (items 5,6, 8, 10, and 11)<br>5. Patient Global Impression of Change (PGI-C)<br><br>• To evaluate the effects of RT001 on Visual Analogue Scale [VAS]<br>• To evaluate the effects of RT001 on speech measurements and Speech Assessment questionnaire<br>• To evaluate the effect of RT001 on a timed 25-feet walk (T25FW)<br>• To evaluate the effects of RT001 on the neurological examination of the Friedreich Ataxia Rating Scale (FARS-Neuro)<br>• To evaluate the effects of RT001 on the activities of daily living of the Friedreich Ataxia Rating Scale (FARS-ADL)<br>• To evaluate the effects of RT001 on SF-36<br>• To evaluate the effects of RT001 on the Neuro-QoL scales (Upper Extremity Function and Lower Extremity Function)<br>• To evaluate the effects of RT001 on the frequency of falls as reported in a fall diary and subject's fear of falling<br>• To evaluate the effects of RT001 on the Patient Global Impression of Change (PGI-C) Scale<br>• To evaluate the effects of RT001 on efficacy assessments after 4 months of treatment<br>• To evaluate the effects of RT001 on efficacy assessments after 8 months of treatment<br>• To evaluate the effects of RT001 on ejection fraction as reported in the Echocardiogram<br>• To evaluate the effects of RT001 on hypertrophy as reported in the Echocardiogram<br>**Optional PK Profile Sub-Study Objective:** |

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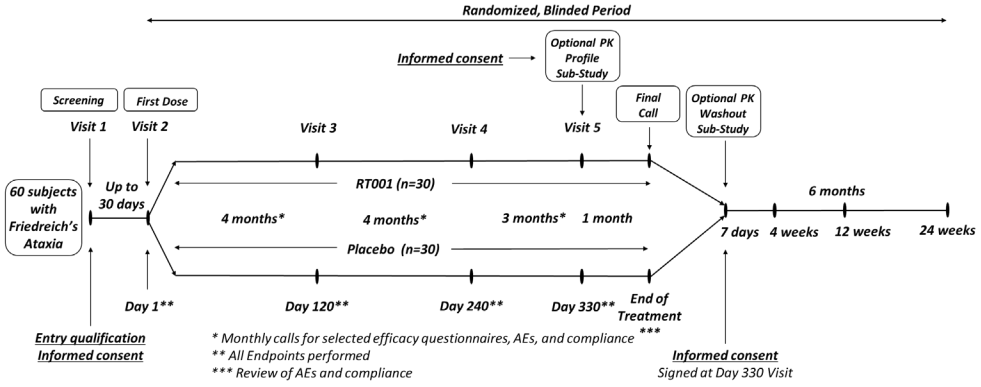
| | The objective of the study is to evaluate the pharmacokinetic (PK) profile of RT001 at steady state.<br>**Optional PK Washout Sub-Study Objective:**<br>The objective of the study is to evaluate the washout blood levels of RT001 after treatment in RT001-006 is completed. |
| **Study Methodology** | This is a randomized, double-blind, placebo-controlled study to evaluate the efficacy, safety, tolerability, in subjects with FRDA following the oral administration of study drug (active or placebo capsules). Subjects will self-administer the study drug orally with meals. While on study drug, subjects are recommended to avoid eating foods high in PUFAs. Subjects will keep food journals from screening through the completion of month 1 on study drug. After month 1, subjects will keep 2-week food journals prior to the next scheduled onsite visit. Subjects (or parents/caregivers, as applicable) will have an initial call and as needed with the diet coach to discuss food journal completion requirements.<br><br>CPET and the T1MW/T25FW should be separated in time to allow the subject to recover sufficiently before doing the next test. CPET should occur in the morning after fasting for a minimum of 2 hours (if the subject has had a meal/snack), with neither caffeine nor nicotine for at least 12 hours before testing. All tests should be done at approximately the same time and order each day. |
| **Study Duration** | Participation is anticipated to require 13 months.<br>• **Screening**: Up to 30 days from start of treatment<br>• **Treatment Period:** treatment for a 12-month period<br>For patients who consent to the Optional Washout Sub-Study Objective, the study duration will be 19 months. |
| **Study Design** | The planned enrollment is 60 subjects randomized 1:1 active: placebo. There are two optional PK sub-studies. Subjects will have the opportunity to consent to these studies at the Day 330 visit or at the time of the first draw.<br>  |
| **Safety Assessments** | Safety assessments include physical and neurologic examinations, vital signs, 12-lead electrocardiogram (ECG) tracing, and clinical laboratory tests (hematology, clinical chemistry, lipid profile, coagulation, and urinalysis) to identify adverse events (AEs). Adverse events will be evaluated for incidence, severity, and relationship to study drug. Any AE ongoing at the End of Treatment (EOT) phone call will be followed to conclusion or until stabilized. |

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| **PK/Biomarker Analysis** | Bioanalytical measurements will be performed to determine the concentration of D2-LA, deuterated arachidonic acid (D2-ARA), H2-LA and nondeuterated arachidonic acid (H2-ARA) in plasma, and D2-LA, D2-ARA H2-LA, and H2-ARA in red blood cells.<br><br>Blood samples will be collected for the plasma measurements as follows:<br>Month 4 (Day 120 ± 30 days): Pre-dose (prior to breakfast)<br>Month 8 (Day 240 ± 30 days): Pre-dose (prior to breakfast)<br>Month 11 (Day 330 ± 30 days): Pre-dose (prior to breakfast)<br><br>Blood samples for the assessments in red blood cells will be collected as follows:<br>Month 4 (Day 120 ± 30 days): Pre-dose (prior to breakfast)<br>Month 8 (Day 240 ± 30 days): Pre-dose (prior to breakfast)<br>Month 11 (Day 330 ± 30 days): Pre-dose (prior to breakfast)<br><br>Additional blood samples will be collected and stored for future biomarker analysis as follows:<br>Day 1: Pre-dose (prior to breakfast)<br>Month 4 (Day 120 ± 30 days): Pre-dose (prior to breakfast)<br>Month 8 (Day 240 ± 30 days): Pre-dose (prior to breakfast)<br>Month 11 (Day 330 ± 30 days): Pre-dose (prior to breakfast)<br><br>Two additional optional PK sub-studies are offered. The first study is a 24hr PK Profile at steady state that will occur immediately before or after the Day 330 visit. Blood samples for this study will be collected as follows:<br>Day 1: Hour -1.0 to -0.5 (pre-breakfast, pre-dose), 0.5 (± 5 min), 1 (± 5 min), 2 (± 10 min), 4 (± 15 min) (pre-lunch), 8 (± 15 min) (pre-dinner), and 12 (± 60 min)<br>Day 2: 24 hours following dosing on Day 1 (± 60 min; pre-breakfast, pre-dose)<br><br>The second optional sub-study is a 6-month study to evaluate the washout blood levels of RT001 after treatment in RT001-006 is completed. Depending on the timing of entry into the sub-study, blood samples for this study will be collected as follows:<br>7 days (± 1 day)<br>4 weeks (± 7 days)<br>12 weeks (± 7 days)<br>24 weeks (± 7 days) |
| **Efficacy Assessments** | Subjects will perform CPET using a recumbent exercise bike at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Peak oxygen consumption and peak work rate will be measured during incremental exercise testing. CPET should occur after fasting (if the subject has had a meal/ snack) for a minimum of 2 hours, with neither caffeine nor nicotine for at least 12 hours before testing. |

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| | Subjects (with or without assistive device) will perform a T1MW and T25FW at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days).<br><br>CPET and the T1MW/T25FW should be separated in time to allow the subject to recover sufficiently before doing the next test. All tests should be done at approximately the same time and order for each visit.<br><br>The FARS-Neuro and clinician-rated CGI scale will be performed at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days) by a qualified physician or health professional who is trained on the use of the tool. The CPET examination should always precede the FARS-Neuro evaluation at all visits.<br><br>A trained health professional will also work with the subject to perform the speech measurement recording at Screening, on Day 1, Day 240 (± 30 days), and Day 330 (± 30 days).<br><br>The FARS-ADL, SF-36, VAS (for Fatigue), Fatigue scale, Neuro-QoL scales (lower extremity function and upper extremity function) and Fall Questionnaire will be completed by the subjects at screening and at the visits on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). The Patient Global Impression of Change (PGI-C) scale and VAS (for Improvement of FA symptoms) will be completed by all subjects at Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). In between the clinic visits, subjects will also be contacted by phone by a study site staff member and asked questions relating to the PGI-C scale, Fatigue scale, Neuro-QoL scales (lower extremity function, and upper extremity function), and questions 8 and 9 of the Fall Questionnaire. These phone 'visits' will take place monthly. A Speech Assessment Questionnaire will also be completed by the subject and parents/caregiver (as applicable) at Screening, on Day 1, Day 240 (± 30 days), and Day 330 (± 30 days).<br><br>Between clinic visits subjects will be asked to keep a daily log (diary) documenting falls, near-falls, stumbles, and fear of falling. |
| **Inclusion Criteria** | <u>To be enrolled into the study, subjects must meet the following requirements:</u><br>1. Male or female 12 to 50 years of age<br>2. Medical history consistent with the symptoms of FRDA at ≤ 25 years of age<br>3. Detection of biallelic pathogenic variants in frataxin gene (*FXN)* (i.e. biallelic repeat expansions of GAA in the disease-causing range, or compound heterozygous for a repeat expansion of GAA in the disease-causing range + an intragenic pathogenic variant, etc.)<br>4. Ambulatory (with or without assistive device) and capable of performing other assessments/evaluations<br>5. Must be able to walk 25 feet during the timed 1-minute walk<br>6. Agree to receive calls from a diet coach regarding completion of electronic food journal<br>7. Sign the informed consent form prior to entry into the study<br>8. Agree to stay locally for the required visit days<br>9. Able to provide the necessary repeated blood samples |

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| **Exclusion Criteria** | Subjects meeting one or more of the following may not enter the study:<br>1. Received treatment with other experimental therapies within the last 30 days prior to the first dose<br>2. Previously participated in the RT001 trial<br>3. Refusal to discontinue fish oils or other oil-based supplements for the duration of the study (Screening until last study procedure completed)<br>4. History of malignancies (other than basal cell carcinomas)<br>5. Inability to complete CPET protocol, as, for example, unable to move the pedals on the exercise bicycle, inability to maintain sufficient RPMs to finish the study, or as judged by the exercise physiologist supervising the testing<br>6. Female who is breastfeeding or has a positive pregnancy test<br>7. Male participant or female participant of childbearing potential, who is sexually active and unwilling/unable to use a medically acceptable and effective double barrier birth control method throughout the study<br>8. Unwilling or unable to comply with the requirements of this protocol, including the presence of any condition (physical, mental, or social) that is likely to affect the subject's ability to return for visits as scheduled<br>9. Clinically significant cardiac abnormalities (e.g., left ventricular ejection fraction [LVEF] < 40% on a recent echocardiogram [obtained within 12 months prior to screening] or clinically unstable arrhythmia) at screening that, in the opinion of the Investigator, would make the subject unsuitable for enrollment<br>10. History of uncontrolled diabetes mellitus (Type 1 or 2)<br>11. Suicidal ideation within the last 12 months as determined by the Columbia-Suicide Severity Rating Scale (C-SSRS)<br>12. History, within the last 2 years, of alcohol abuse, significant mental illness, or physical opioid dependence |
| **Food Journal requirement** | While on study drug, subjects are recommended to avoid eating foods high in PUFAs. Subjects will keep food journals from screening through the completion of month 1 on study drug. After month 1, subjects will keep 2-week food journals prior to next scheduled onsite visit. Subjects (or parents/caregivers, as applicable) will have an initial call and as needed with the diet coach to discuss food journal completion requirements. |
| **Prohibited Medications** | Supplements that contain EPA or DHA are prohibited. Oil-based supplements like Fish oil, Cod Liver oil, Edible algae oil and Flax oil are also prohibited. |
| **Statistical methods** | Demographic data will be presented using descriptive statistics (e.g., mean, standard deviation, median, and range). Exposure to study drug and reasons for discontinuation will be tabulated. Safety variables will be tabulated and presented for all subjects who receive study drug or placebo capsules. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs will be presented by system organ class and preferred term. Adverse events will be presented by severity and relationship to |

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| | study drug. Changes from Baseline in clinical laboratory parameters and vital signs will be summarized across time. Shift tables will be provided for selected laboratory parameters. Physical examination results will be presented in listings.<br>Change from baseline (CFB) will be analyzed by comparing the change between treatment groups using a mixed model with repeated measures (MMRM). The MMRM will compare the estimated change from baseline between treatments for the primary endpoint. This analysis will assess whether there is a difference in estimated CFB between active and placebo groups at 11 months.<br>The primary analysis of percent change from baseline to 11 months in MVO2 as measured by CPET will use the ITT methodology and an MMRM methodology with a random slope and intercept for each individual.<br><br>All secondary and exploratory endpoints CFB values will be analyzed using the MMRM Responder analyses will be run on MVO2 (primary), GST, T1MW, Peak Workload, mFARS, and CGI-C (functional assessment). The PK of D2-LA, D2-ARA, H2-LA, and H2-ARA will be characterized in terms of minimum and maximum exposure at steady state.<br>For the primary analysis of peak workload, group sample sizes of 30 and 30 achieve over 99% power to reject the null hypothesis of equal means when the population mean difference is $\mu 1 - \mu 2 = 7.5 - -8.3 = 15.8$ with a standard deviation for both groups of 11.1 and with a significance level (alpha) of 0.05 using a two-sided two-sample equal-variance t-test. |
| **Safety Monitoring** | The blinded safety and efficacy data will be reviewed by the medical monitor on an ongoing basis. A Data Safety and Monitoring Board (DSMB) will be established to evaluate events according to the DSMB Charter. The safety and efficacy review data will not be made available to the Principal Investigators unless changes need to be made to the protocol for safety reasons. |

## Table 1: Schedule of Events

| Assessments | Study Period | Screening | | Study Drug Administration | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Clinic Visit No. | 1 | 2 | Phone Call | 3 | Phone Call | 4 | Phone Call | 5 | End of Treatment Phone Call |
| | Study Day | Up to 30 Days | Day 1 | Days 30,60, 90 (± 7 days) | Day 120 (± 30 days) | Days 150, 180, 210 (± 7 days) | Day 240 (±30 days) | Days 270, 300 (± 7 days) | Day 330 (±30 days) | 4 weeks after Day 330 visit (+ 7 days) |
| Informed Consent | | X | | | | | | | | |
| Inclusion/Exclusion Criteria | | X | X | | | | | | | |
| Demographics/Medical History | | X | X | | | | | | | |
| Columbia-Suicide Severity Rating Scale | | X | | | | | | | | |
| Echocardiogram[a] | | X | | | | | | | X | |
| Serum Pregnancy Test[b] | | X | | | | | | | | |
| Cardio-Pulmonary Exercise Test (CPET)[c] | | X | X | | X | | X | | X | |
| T1MW and T25FW | | X | X | | X | | X | | X | |
| Physical Exam | | X | X | | X | | X | | X | |
| FARS-Neuro and clinician-rated CGI[d] | | X[d] | X[d] | | X | | X | | X | |
| Speech Measurement Recording | | X | X | | | | X | | X | |
| Subject completed Speech Assessment Questionnaire | | X | X | | | | X | | X | |
| FARS ADL, SF36, VAS[e] | | X[e] | X[e] | | X | | X | | X | |
| Subject completed PGI-C[f], Fatigue scale, and Neuro-QoL scales | | X[f] | X[f] | X | X | X | X | X | X | |
| Fall Questionnaire | | X | X | X[g] | X | X[g] | X | X[g] | X | |
| Vital Signs (including weight) | | X[h] | X | | X | | X | | X[h] | |
| 12-Lead ECG[i] | | X | X[i] | | X | | X | | X | |
| Urine Pregnancy Test[b,l] | | | X | | X | | X | | X | X[l] |
| Urinalysis | | X | | | | | | | X | |
| Safety Laboratory Tests[i,j,k] | | X | X[i] | | X | | X | | X | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Lipid Profile[i,k] | X | X[i] | | X | | X | | X | |
| Randomization | | X | | | | | | | |
| In-Clinic Drug Administration | | X | | X | | X | | X | |
| Study Drug Dispensing[m] | | X | | X | | X | | X | |
| Perform Drug Accountability[n] | | | | X | | X | | X | X[n] |
| PK Sampling | | | | X | | X | | X | |
| Biomarker Sampling | | X | | X | | X | | X | |
| Monitor AE/Concomitant Medication | | X | X | X | X | X | X | X | X[o] |
| Fall diary | ← | | | | | | | → | |
| Food Journal | X[p] | X[q] | | X[p] | | X[p] | | X[p] | |

[a] Does not need to be performed in subjects who had echocardiogram done in the last 12 months before screening.
[b] Serum and Urine Pregnancy Tests to be performed on all females, unless there is documented evidence confirming they are not of childbearing potential.
[c] The cardio-pulmonary exercise test (CPET) should occur in the morning after fasting for a minimum of 2 hours, with neither caffeine nor nicotine for at least 12 hours before testing. All tests should be done at approximately the same time and order for each visit. The CPET should always precede the FARS-Neuro evaluation at all visits.
[d] For Clinician Rated CGI, only question 1 will be answered at Screening and Day 1
[e] Only VAS (for Fatigue) will be performed at Screening and Day 1. VAS (for Improvement of FA Symptoms) will not be performed at Screening or Day 1.
[f] Patient Global Impression of Change (PGI-C) to be performed Day 30 and onwards only. Not to be performed at Screening and Day 1.
[g] Only questions 8 and 9 of the Fall Questionnaire will be answered over phone visits
[h] At Screening and Day 330, height will be collected, and body mass index (BMI) will be calculated.
[i] Safety labs, Lipid Profile and ECG do not have to be repeated at Day 1 if the screening and Day 1 visit is within 7 days (or less) of each other.
[j] Safety laboratory tests include chemistry, hematology, and coagulation.
[k] Safety laboratory tests and lipid profile should be performed in a fasting state.
[l] Urine Pregnancy test to be performed by subject at home on the day of the EOT phone call (before the call and after the last dose).
[m] The study drug for the first 4 weeks of dosing will be dispensed at the study sites. Study Drug for the subsequent dosing months may be shipped to the subject or site from a drug depot or site, depending on the dispensing schedule.
[n] Site to perform drug accountability with subject on the EOT call. Subject to return unused study drug/bottles to the site.
[o] Any AE ongoing at EOT will be followed to conclusion or until stabilized.
[p] The Diet Coach will have an initial call and as needed with the subjects (or parents/ caregivers as applicable) to discuss food journal requirement.
[q] The food journal has to be completed through end of month 1, and every 2 weeks prior to next onsite visit.

## Table 1.1: Optional PK Profile Sub-Study PK Schedule of Events

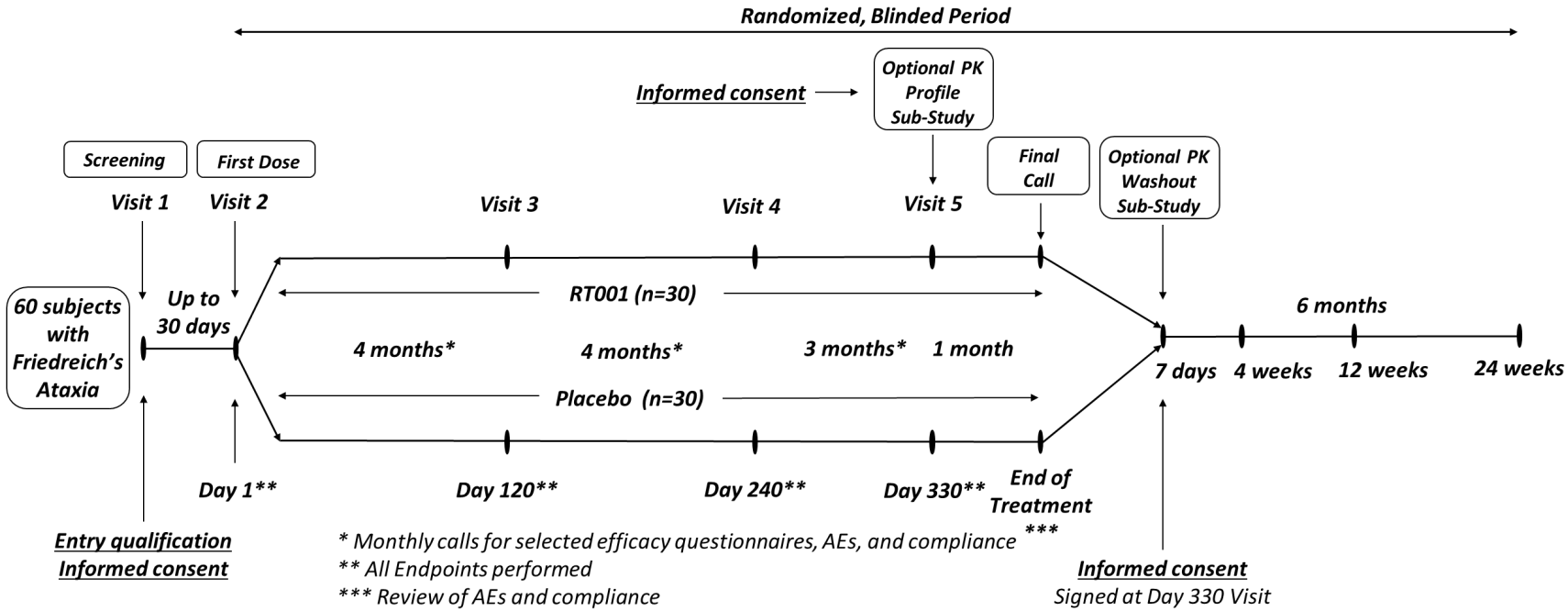
| **Assessments** | **Pharmacokinetic Profile of RT001 at Steady State in Subjects with Friedreich's Ataxia Who Participated in Study RT001-006** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Study Time** | **-1 Hour to -30 Minutes (± 5 min)** | **30 Minutes (± 5 min)** | **1 Hour (± 5 min)** | **2 Hours (± 10 min)** | **4 Hours Before lunch (± 15 min)** | **8 Hour Before dinner (± 15 min)** | **12 Hour Post dinner (± 60 min)** | **24 Hour Day 2 (± 60 min)** |
| | **Notes** | **Pre-breakfast, pre-dose** | **Post-breakfast, post-dose** | **Post-breakfast, post-dose** | **Post-breakfast, post-dose** | **Pre-lunch, post-dose** | **Post-lunch, post-dose** | **Post-dinner, skip evening dose** | **Pre-breakfast, pre-dose** |
| PK Sampling | | X | X | X | X | X | X | X | X |

## Table 1.2: Optional Washout Sub-Study Schedule of Events

| **Assessments** | **Washout Blood Levels of Subjects Who Have Completed Treatment in RT001-006** | | | | |
|---|---|---|---|---|---|
| | **Study Day** | **Day 7 (± 1 day)** | **Week 4 (± 7 days)** | **Week 12 (± 7 days)** | **Week 24 (± 7 days)** |
| PK Sampling | | X[a,b] | X[a,b] | X[a,b] | X[a,b] |

[a] Draw can be performed onsite or remotely by a mobile phlebotomist
[b] Subjects who complete RT001-006 are eligible to participate in any part of the PK sub-study.

# 3. TABLE 2: STUDY SCHEMATIC



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## TABLE 4: TABLE OF CONTENTS

# 5. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

**Table 5: Abbreviations and Specialist Terms**

| Abbreviation or Specialist Term | Explanation |
|---|---|
| ADL | Activities of Daily Living |
| AE | Adverse event |
| ALT | Alanine transaminase |
| AST | Aspartate transaminase |
| BID | Twice a day |
| BMI | Body mass index |
| BUN | Blood urea nitrogen |
| CDC | Centers for Disease Control |
| CGI | Clinical Global Impression |
| $CO_2$ | Carbon dioxide |
| CPET | Cardio-Pulmonary Exercise Testing |
| CRF | Case Report Form |
| CRO | Clinical research organization |
| CSR | Clinical study report |
| C-SSRS | Columbia-Suicide Severity Rating Scale |
| D-PUFAs | Deuterated polyunsaturated fatty acids |
| $D_2$-ARA | Deuterated arachidonic acid |
| $D_2$-LA | Deuterated linoleic acid |
| DSMB | Data Safety Monitoring Board |
| ECG | Electrocardiogram |
| eCRF | Electronic case report form |
| FARS | Friedreich's ataxia rating scale |
| FARS-ADL | Activities of daily living of the Friedreich Ataxia Rating Scale |
| FARS-Neuro | Neurological examination of the Friedreich Ataxia Rating Scale |
| FDA | Food and Drug Administration |
| FeS | Iron sulfur |
| FRDA | Friedreich's ataxia |
| FXN | Frataxin gene |
| GCP | Good Clinical Practice |

| | |
|---|---|
| GI | Gastrointestinal |
| GLP | Good Laboratory Practice |
| GRAS | Generally recognized as safe |
| GST | Global Statistical Test |
| $H_2$-ARA | Nondeuterated arachidonic acid |
| $H_2$-LA | Nondeuterated linoleic acid |
| HBsAg | Hepatitis B surface antigen |
| HDL | High density lipoprotein |
| HED | Human equivalent dose |
| HIPAA | Health Insurance Portability Assurance Act |
| HIV | Human immunodeficiency virus |
| HNE | 4-hydroxynonenal |
| HSP60 | Heat shock protein 60 |
| ICF | Informed consent form |
| ICH | International Conference for Harmonization |
| IND | Investigational New Drug |
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| ITT | Intent-To-Treat |
| IV | Intravenous |
| KIE | Kinetic isotope effect |
| LA | Linoleic acid |
| LDL | Low density lipoprotein |
| LVEF | Left ventricular ejection fraction |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mFARS | Modified Friedreich's Ataxia Scale |
| mITT | modified Intent-To-Treat |
| mRNA | Messenger RNA |
| MVO2 | Maximum rate of oxygen consumption |
| NOAEL | No observed adverse effect level |
| PBMC | Peripheral blood mononuclear cell |
| PD | Pharmacodynamic |
| PGI-C | Patient Global Impression of Change |

| | |
|---|---|
| PK | Pharmacokinetic |
| PT | Prothrombin time |
| PTT | Partial thromboplastin time |
| PUFAs | Polyunsaturated fatty acids |
| QA | Quality Assurance |
| QC | Quality Control |
| QD | Once a day |
| RBC | Red blood cell |
| ROS | Reactive oxygen species |
| SAE | Serious adverse event |
| SOC | System organ class |
| SOP | Standard operating procedure |
| SUSAR | Suspected unexpected serious adverse reaction |
| T25FW | Timed 25-foot walk |
| T1MW | Timed 1-minute walk |
| TEAE | Treatment-emergent adverse event |
| TID | Three times a day |
| TMF | Trial Master File |
| ULN | Upper limit of normal |
| US | United States |
| VAS | Visual Analogue Scale |
| $VO_2$ MAX | Maximum rate of oxygen consumption |
| WBC | White blood cell |
| WHODD | World Health Organization Drug Dictionary |
| WOCBP | Women of childbearing potential |

# 6. INTRODUCTION

## 6.1. Friedreich's Ataxia

Friedreich's ataxia (FRDA), a syndrome of ataxia in all 4 limbs with an onset of symptoms between late childhood and early adolescence, is a model of significant mitochondrial dysfunction with excess chronic oxidative damage leading to selective cell death and significant neurologic damage (Santos et al., 2010; Armstrong et al., 2010). It is characterized by neurodegeneration of the dorsal root ganglia with loss of large sensory neurons and the posterior columns, and by degeneration in the spinocerebellar and corticospinal tracts in the spinal cord with cerebellar involvement (Santos et al., 2010). While relatively rare, it is the most common of the inherited ataxias with prevalence of 1:50,000 in the United States (US) (Armstrong et al., 2010; Santos et al., 2010). Other disease findings may include scoliosis, diabetes, incontinence, cardiomyopathy, optic atrophy, and hypoacusis with median life expectancy of 35 years. Physiologically, FRDA is characterized by mitochondrial dysfunction and inefficient oxidative metabolism with three particular findings: intracellular iron deposits; a deficit in the mitochondrial iron-sulfur (FeS) cluster containing enzymes such as aconitase and respiratory chain Complexes I-III; and the presence of markers of oxidative damage in the blood and urine (Martelli et al., 2012).

The genetic basis of FRDA is a recessive GAA trinucleotide expansion mutation of the first intron in the frataxin gene (FXN). The normal frataxin gene has ≤ 40 GAA repeats, while FRDA subjects have increasing and varying GAA repeats. This expansion leads to significantly decreased protein expression levels (5% to 30% of normal) of the frataxin protein (Santos et al., 2010; Martelli et al., 2012). The low levels of frataxin cause dysregulation of normal iron levels resulting in an insufficient supply of iron to the FeS complexes, the primary cause of inefficient mitochondrial energy generation. The suboptimal delivery of iron to the FeS complexes results in a number of secondary potentially toxic reflexive mechanisms such as an excess of free $Fe^{+2}$, which in turn catalyzes $HO^{\bullet}$ (hydroxy radical) production from $H_2O_2$ that leads to widespread lipid peroxidation (Foury & Cazzalini, 1997; Qian & Buettner, 1999). The chronic reactive oxygen species (ROS) generation overwhelms the intramitochondrial and intracellular ROS quenching systems leading to significant oxidative damage, inefficient power generation, widespread cellular dysfunction, and premature cell death (Armstrong et al., 2010; Guéraud et al., 2010).

## 6.2. Oxidative Stress and Neurodegenerative Disease

A great deal of evidence exists that demonstrates the involvement of oxidative stress in the pathology of neurological disorders. Moreover, the vulnerability of the central nervous system to ROS-mediated injury is well established since neurons consume large amounts of oxygen, the brain has many areas containing high iron content, and neuronal mitochondria generate large

amounts of hydrogen peroxide. Furthermore, neuronal membranes are rich in polyunsaturated fatty acids, which are particularly susceptible to oxidative stress. The biological roles of products produced by lipid peroxidation have received much attention, not only for their pathological mechanisms associated with neurological disorders, but also for their practical clinical applications as biomarkers. Reactive oxygen species are likely to play a pathophysiological role in many neurological disorders, including Alzheimer's disease, Down syndrome, Parkinson's disease, and stroke.

The brain is extremely sensitive to oxidative damage for several reasons. The brain consumes an inordinate amount of oxygen (around 20%), particularly when considering the fact that the brain accounts for only 2% of body weight (Halliwell, 2006). A major reason for the high $O_2$ uptake is the vast amounts of ATP needed to maintain neuronal intracellular ion homeostasis in the face of ion channels need for action potentials and neurosecretion. Additionally, several brain areas, including the substantia nigra, caudate nucleus, putamen, and globus pallidus, are rich in iron content (Zecca et al., 2004). It is generally accepted that iron accumulates in the brain of older individuals, and iron ions that are released following brain damage can catalyze free radical reactions. The brain is a source of ROS. Complex I-dependent hydrogen peroxide generation in brain mitochondria is greater than that in skeletal muscle mitochondria (Malinska et al., 2009). Lastly, neuronal membranes are rich in polyunsaturated fatty acids (PUFAs), particularly arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) (Chen et al., 2008). These PUFAs are particularly vulnerable to oxidative stress due to their degree of unsaturation (multiple double bonds). For these reasons, neural cells are more susceptible to oxidative damage when compared to other body tissues.

The mechanism of ROS production differs in each neurological disorder; excess amounts of oxidized lipids causing neurological degeneration may be the result of excessive generation of these compounds, reduced clearance, or some combination of the two processes. These differences provide multiple targets for therapeutic intervention. However, any intervention that results in less lipid peroxidation is likely to exhibit some therapeutic benefit in neurological degeneration. In FA, the cause of excessive ROS is the excess production that accompanies low levels of expression of the iron chaperon protein frataxin, causing oxidation stress from Fenton reaction catalysis of free-iron induced lipid peroxidation.

## 6.3. Non-Clinical Rationale Supporting RT001 Development for FRDA

RT001 is a site-specific (C11) di-deutero synthetic homologue of linoleic acid (LA) ethyl ester. It has been established that substitution of hydrogen with deuterium at specific bis-allylic sites, as in RT001, decreases the production of lipid peroxidation products in yeast models of oxidative stress (Hill et al., 2011; Hill et al., 2012; Grazia Cotticelli et al., 2013; Abeti et al., 2015; Andreyev et al., 2015). *In vitro* yeast, murine, and human models of FRDA also indicated that RT001 free acid managed the oxidative stress associated with increased iron (Grazia Cotticelli et

al., 2013; Abeti et al., 2015). The significance of the results with FRDA model systems is 3-fold. First, oxidative stress in FRDA has been difficult to measure, and the protective effects of deuterated polyunsaturated fatty acids (D-PUFAs) in the FRDA cell models indicate not only that there is oxidative stress in these models, but also emphasize the importance of lipid peroxidation damage. Second, results further support the proposal that reinforcing essential PUFAs with isotopic replacement decreases lipid peroxidation and decelerates toxic cellular cascades. Third, the results suggest that dietary dosed D-PUFAs such as RT001, which are easily distributed to all the target tissues, might be an effective therapeutic strategy for the treatment of FRDA. The chemical basis for this decrease in lipid peroxidation is the kinetic isotope effect (KIE). KIE is related to the difference in molecular weight, substitution of deuterium for hydrogen (with a 100% in increase in molecular weight) exhibits one of the largest KIEs observed. The KIE of RT001 vs. LA has been well documented (Hill et al., 2012, Lamberson et al., 2014).

Given the deleterious effects of frataxin mutations on the mitochondrial electron transport chain, and the presence of mitochondrial iron accumulation in FRDA, the PUFA-rich inner mitochondrial membranes are the primary sites of autoxidative damage. In H9C2 myoblasts, treatment with t-ButOOH caused both respiratory inhibition and increased membrane leak. RT001 free acid protected mitochondrial function from stress caused by t-ButOOH; maximal respiration was preserved and membrane leakage was diminished (Andreyev et al., 2015).

## 6.4. Clinical Rationale Supporting RT001 Development for FRDA

RT001-002 was a randomized, double blind, comparator controlled, 28-day clinical study with 2 cohorts of 9 subjects each randomized 2:1 to RT001 or linoleic acid control in identical gel capsules. Cohort 1 received 1.8 g/day (2 capsules) and Cohort 2 received 9.0 g/day (10 capsules) with meals. All subjects were followed for 28 days with a 30-day safety follow up. The primary endpoint was safety, tolerability, and pharmacokinetics. The secondary endpoints were timed 25-foot walk (T25FW) test and Friedreich Ataxia Rating Scale (FARS) neurologic scale measurement comparing baseline to test results on Day 28. Exploratory endpoints included changes in pharmacodynamic biomarkers, multi-axis movement measurement of the lower extremity during the T25FW test, and measurement of exercise capacity using a Cardio-Pulmonary Exercise Testing (CPET) protocol to measure peak workload and maximum rate of oxygen consumption ($VO_2$ MAX) when performed on a recumbent bicycle with full pulmonary metabolic measurements.

A total of 19 subjects were enrolled in RT001-002 (13 subjects in the RT001 group and 6 subjects in the comparator group). Of the 19 enrolled subjects, 17 subjects completed the study. The two subjects who withdrew from the study were in the RT001 group.

Plasma deuterated linoleic acid ($D_2$-LA) concentrations rose over the course of the 28-day study in both cohorts and appeared to be approaching a steady state by the Day 28 dosing interval. Mean plasma $D_2$-LA concentrations on Day 28 fluctuated slightly (less than 2-fold) during the 12 or 24 h dosing interval, and then fell slowly, with a mean terminal half-life of 42.3 ± 9.8 h in Cohort 1 and 30.3 ± 18.3 h in Cohort 2 (Table 6, Table 7). When the daily dose was increased 5-fold from 1.8 g to 9.0 g, the $AUC_{0\text{-}24h}$ on Day 28 rose from 1527 ± 445 ug•h/mL to 6800 ± 918 ug•h/mL (approx. 4-fold).

**Table 6. Summary of $D_2$-LA PK Parameters in Plasma on Day 1**

| | | **Cohort 1 1.8 g QD** | | | **Cohort 2 4.5g BID** | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **units** | n | Mean | SD | n | Mean | SD |
| **$C_{max}$** | **ug/mL** | 6 | 35.9 | 9.8 | 7 | 58.6 | 16 |
| **$T_{max}$** | **h** | 6 | 6.1 | - | 7 | 8.0 | -- |
| **$AUC_{last}$** | **ug h/mL** | 6 | 520 | 86 | 7 | 912 | 251 |

Median shown for $T_{max}$. On Day 1 $AUC_{last}$ was 24h

**Table 7. Summary of D2-LA PK Parameters in Plasma on Day 28**

| | | Cohort 1 1.8 g QD | | | Cohort 2 4.5g BID | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **units** | n | Mean | SD | n | Mean | SD |
| **$C_{max}$** | **ug/mL** | 6 | 80.8 | 24.6 | 5 | 359.9 | 31.6 |
| **$T_{max}$** | **h** | 6 | 7.9 | - | 5 | 6.0 | - |
| **$C_{min}$** | **ug/mL** | 6 | 26.0 | 10.1 | 5 | 86.2 | 43.7 |
| **$T_{min}$** | **h** | 6 | 71.8 | - | 5 | 72.0 | - |
| **$AUC_{0-12h}$** | **ug h/mL** | 6 | 809 | 269 | 5 | 3400 | 459 |
| **$AUC_{0-24h}$** | **ug h/mL** | 6 | 1527 | 445 | 5 | 6800[a] | 918 |
| **$AUC_{last}$[b]** | **ug h/mL** | 6 | 3345 | 950 | 5 | 13854 | 2899 |
| **$t_{1/2}$** | **h** | 5 | 42.3 | 9.77 | 4 | 30.3 | 18.30 |

Medians shown for $T_{max}$, $T_{min}$

a – For comparison of the two dosing regimens, the $AUC_{0-24h}$ shown here is the $AUC_{0-24h}$ at steady state (= 2 * $AUC_{0-12}$) for the BID regimen

b – $AUC_{last}$ was 72 h after the last dose in all subjects

The CPET testing change between baseline and Day 28 showed an improvement in peak workload of +0.16 watts/kg (25.7%) in the subjects on all doses of study drug (n=10) vs. comparator (n=6). The reported p value in post hoc analysis was p=0.008 (Mann-Whitney Rank Sum test). This modified Intent-To-Treat (mITT) analysis excluded 2 subjects unable to either start or complete the test, and full Intent-To-Treat (ITT) analysis including the two subjects, had a p= 0.021. Similarly, the study showed a strong trend from baseline to Day 28 of therapy in peak oxygen consumption ($VO_2$ MAX). The median change from baseline for comparator vs. RT001 was -0.02 L/min vs. 0.14 L/min; respectively, p=0.116. Assessment on the neurological examination of the Friedreich Ataxia Rating Scale (FARS-Neuro) scoring showed a trend in improvement between baseline and Day 28 of treated vs. placebo patients (a decrease of 1.95 FARS points, p= 0.348 ITT). This result was on top of a decrease of 2.8 FARS points in the placebo group, (i.e. a 4.75 FARS point decrease from baseline for the treated group), highlighting the robust placebo effect in the FARS-Neuro measure that has been documented elsewhere (Zesiewicz, 2017). Expected change in FARS-Neuro for one month of dosing based on extensive natural history data (Patel et. al., 2016) would be ~ +0.25 FARS points. The average stride speed $(\text{stride time})^{-1}$ during the T25FW, measured with an electronic motion sensor, improved 7% (p=0.15 ITT). Traditionally measured $T25FW^{-1}$ (via stopwatch) showed no signal of significance (p=0.88).

The relatively small size and short duration of the trial necessitates caution in over-interpretation of efficacy results. Nevertheless, clear correlations between the various parameters measured

cross-sectionally across all patient encounters can be seen in RT001-002. Comparisons of peak workload, $VO_2$ MAX, FARS-Neuro Score, and T25FW show consistent correlation. This indicates that these are all legitimate measures of disease severity, and correlative to traditional measures such as FARS neuro, which may be less consistent and predictable than CPET-determined peak workload and $VO_2$ MAX.

## 6.5. Non-Clinical Safety Summary

Refer to the Investigator Brochure for a summary of the nonclinical studies conducted with RT001. There was no adverse finding in any parameter in Good Laboratory Practice (GLP) dietary toxicity rat studies of RT001 up to 26-weeks at the highest doses tested, averaging 452 mg/kg (401 and 502 mg/kg in males and females; respectively).

Long-term increased intake of dietary fats, including LA, can lead to obesity when consumed above normal ranges. As a result, obese rats are no longer normal, and this state of physiological dysregulation has a confounding effect on the interpretation of direct toxicological effects (Heden et al., 2014). To avoid the confounding effects of obesity, the LA content in rat studies were based on physiologic levels of PUFAs. Further dose escalation of RT001 in rat studies poses a challenge as higher doses result in polyunsaturated fat intake in excess of accepted healthy diet values. This would lead to a study in elevated fat intake, elevated PUFA intake, and altered caloric density, rather than a clear study of RT001 effects. In our studies at the highest dose tested, the total fat made up 14% of the subject dietary calories, the recommended maximum level for rats (National Academy of Sciences, 1995), and RT001 made up 100% of the LA component.

In animal studies, RT001 was found to be equivalent to, and biologically interchangeable with normal dietary LA as a sole source (100% replacement) of LA, indicating that the substitution of hydrogen with deuterium does not interfere with the normal biology of the fat. If this was not the case, essential lipid deficiency diseases (Burr & Burr, 1929 and 1930) would be observed in the treated populations. In addition, LA is recognized by the Food and Drug Administration (FDA) as generally recognized as safe (GRAS), and has no upper limit under the GRAS designation. Additionally, IntraLipid® is approved as an IV drug at doses up to 93 g/day LA (2.5 g/kg/day) based on a 60-kg person (IntraLipid Prescribing Information, 2006) representing a 16.3-fold safety margin over the dose of 5.7 g/day.

## 6.6. Clinical Safety Summary

In clinical study RT001-002, one subject in cohort 2 (9g/day dose group) dropped from the study on Day 3 after experiencing severe oily diarrhea (steatorrhea). The subject was hospitalized for overnight observation. The symptoms resolved spontaneously within several hours and there were no signs of gastrointestinal (GI) malabsorption. The subject had a remarkably low body mass at 38.9 kg, suggesting that care must be observed at the highest dose in low body mass

subjects. The subject did not continue with the trial and another subject was recruited. This event was judged to be severe and probably related to study drug. Another subject withdrew due to an AE of depression. Diarrhea was reported in 3 additional subjects in the RT001 treatment group and 1 subject in the comparator treatment group of cohort 2. These events were assessed by the investigators as mild and possibly related to study drug. The other treatment-emergent adverse events (TEAEs) were assessed by the Investigators as mild (grade 1) or moderate (grade 2) in severity and unrelated to study drug. The TEAEs are shown by System Organ Class (SOC) and Preferred Term in Table 8.

**Table 8 TEAEs by System Organ Class and Preferred Term (Safety Population)**

| System Organ Class / Preferred Term | Cohort 1 | | Cohort 2 | |
|---|---|---|---|---|
| | RT001 (N=6) n(%) | Comparator (N=3) n(%) | RT001 (N=7) n(%) | Comparator (N=3) n(%) |
| **Subjects Reporting at Least One TEAE** | **5 (83.3%)** | **2 (66.7%)** | **5 (71.4%)** | **2 (66.7%)** |
| **Gastrointestinal disorders** | **2 (33.3%)** | **0 (0.0%)** | **5 (71.4%)** | **1 (33.3%)** |
| Diarrhea | 0 (0.0%) | 0 (0.0%) | 4 (57.1%) | 1 (33.3%) |
| Abdominal discomfort | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Abdominal distension | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Abdominal pain | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| Constipation | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| Feces discolored | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| Nausea | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| Steatorrhea | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| **Infections and infestations** | **1 (16.7%)** | **2 (66.7%)** | **0 (0.0%)** | **0 (0.0%)** |
| Conjunctivitis viral | 0 (0.0%) | 1 (33.3%) | 0 (0.0%) | 0 (0.0%) |
| Sinusitis | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Upper respiratory tract infection | 0 (0.0%) | 1 (33.3%) | 0 (0.0%) | 0 (0.0%) |
| **Nervous system disorders** | **2 (33.3%)** | **1 (33.3%)** | **0 (0.0%)** | **0 (0.0%)** |
| Dysgeusia | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Sciatica | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Tremor | 0 (0.0%) | 1 (33.3%) | 0 (0.0%) | 0 (0.0%) |
| **Injury, poisoning and procedural complications** | **1 (16.7%)** | **1 (33.3%)** | **0 (0.0%)** | **0 (0.0%)** |
| Facial bones fracture | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Muscle strain | 0 (0.0%) | 1 (33.3%) | 0 (0.0%) | 0 (0.0%) |

| System Organ Class / Preferred Term | Cohort 1 | | Cohort 2 | |
|---|---|---|---|---|
| | RT001 (N=6) n(%) | Comparator (N=3) n(%) | RT001 (N=7) n(%) | Comparator (N=3) n(%) |
| **Respiratory, thoracic and mediastinal disorders** | **1 (16.7%)** | **0 (0.0%)** | **1 (14.3%)** | **0 (0.0%)** |
| Nasal discomfort | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Oropharyngeal pain | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| Rhinorrhea | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| **Blood and lymphatic system disorders** | **1 (16.7%)** | **0 (0.0%)** | **0 (0.0%)** | **0 (0.0%)** |
| Anemia | 1 (16.7%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| **Cardiac disorders** | **0 (0.0%)** | **0 (0.0%)** | **0 (0.0%)** | **1 (33.3%)** |
| Palpitations | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (33.3%) |
| **Psychiatric disorders** | **0 (0.0%)** | **0 (0.0%)** | **1 (14.3%)** | **0 (0.0%)** |
| Depression | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |
| **Skin and subcutaneous tissue disorders** | **0 (0.0%)** | **0 (0.0%)** | **1 (14.3%)** | **0 (0.0%)** |
| Pruritus | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 0 (0.0%) |

In addition to RT001-002, RT001 is currently in use in 19 subjects with infantile neuroaxonal dystrophy in the U.S. The study enrollment is complete, and subjects continue to participate in the extension trial. No serious adverse or unexpected drug-related events have been reported in the study thus far.

RT001 is also being administered in several Expanded Access studies. These Expanded Access studies have been implemented when a plausible mechanism for RT001 therapeutic benefit is considered. The Expanded Access studies have served to support PK, efficacy, and safety data while generating experience in neurodegenerative conditions that might later warrant a formal study in a treatment protocol. Refer to the Investigator Brochure, for additional information of RT001 Sponsor clinical studies and Expanded Access studies.

At the present stage of development, the risk of RT001 seems acceptable considering the potential benefit of RT001 treatment for patients diagnosed with Friedreich's ataxia. Based on these observed gastrointestinal disorder adverse events, we believe that a maximal tolerated dose of 9.0 g/d may have been reached and steps are being taken in the current protocol to address this issue by reducing the dose to 5.76 g/d and dividing the dose between 2 or 3 meals. Additional adjustment possibilities have been added to the protocol to correct for diarrhea-related adverse events for low body mass adults and children.

## 6.7. Inclusion of Pediatric Subjects of 12 Years and Older in the Current Protocol

The decision to include pediatric subjects with FRDA in a clinical trial requires per the regulations a careful analysis of the risks and potential benefits to the subjects. Inclusion of pediatric subjects also involves an analysis of whether or not the subjects would be representative of the population of subjects under study, and whether or not the pediatric subjects would be able to comply with the protocol requirements.

RT001 is a deuterated formulation of linoleic acid, the predominant fatty acid in several approved drug products for IV nutritional supplementation, including the widely used IntraLipid™. Pediatric subjects in the clinical trials that led to the approval of the nutritional supplementation drugs containing linoleic acid constituted more than half of all enrolled patients. Most adverse reactions were related to the route of administration than to the drug itself; the warning for use in pediatric patients limiting use to less than 3g/kg/day was related to a side effect that was clearly linked to IV administration. RT001 is planned for oral administration only, and the maximum daily dosage will be 8.64 g/day for 30 days followed by 5.76g/day. The average weight of a 12 to 14-year old patient is approximately 40 kg. Therefore, the maximum dosage administered orally in our clinical trial will be 0.216 g/kg/day, far less than the maximum recommended IV dosage for pediatric patients using IntraLipid. The use of RT001 in pediatric patients is likely to be safe, as evidenced by the many years of safe use of the nutritional supplements in this age group. In the clinical trials of RT001 in adults, steatorrhea has been the only significant adverse reaction, and this appears to be easily manageable with dose adjustment.

The chief benefit of including pediatric patients in clinical trials with FA include the potential for halting or reversing disease progression. The early age of onset of FA, its most rapid progression in the first years after diagnosis, and its tendency to produce most severe disability in patients who present earliest in life, all necessitate the development of an effective treatment that is applicable to pediatric patients. If RT001 is shown to be beneficial in treating FA, pediatric patients are likely to be the greatest beneficiaries of this therapy.

RT001 is currently in use in a pediatric study with 19 patients with infantile neuroaxonal dystrophy aged between 18 months and 10 years of age. Each patient is receiving RT001 at a dose of 1 capsule TID (2.88 g/day). At present, no adverse drug effects have been identified or reported.

In previous clinical trials of FA patients, pediatric age subjects have been found to be cooperative and important contributors to observational studies (Sival et al. 2011, Patel et al. 2016). The requirement for cardiopulmonary exercise testing (CPET) for determination of the primary endpoint in the clinical trial with RT001 will require subject cooperation which exceeds the capacity of pediatric patients of younger age. Previous observational studies of pediatric patients with FA that required the performance of CPET have limited the use of CPET in the

younger patients for this reason. Pharmacological trials have had lower age limits of 11 years (Artuch et al. 2002), or 16 years (NCT02367014, NCT02255435). Retrotope believes that the enrollment of FA patients 12 years old and above strikes the optimal balance between risk, benefits, and practicality for the present clinical trial.

## 6.8. Potential Risks and Benefits to Human Subjects

FA is a rare, inherited recessive disorder characterized by progressive degenerative changes, in particular to mitochondria, which lead to morbidity and mortality at a young age. There is currently no approved therapy for this condition. RT001 offers a novel yet specific approach to mitigation of the harmful oxidative attack on lipids of mitochondrial membranes, which has potential benefit if the hypothesis is proven in this and subsequent efficacy studies. RT001 is based on a common, essential dietary sourced fatty acid, LA. The slight modification to the chemical structure of LA, the site-specific replacement of 2 hydrogen atoms with 2 deuterium atoms, does not impact any of the key properties and fate of the lipid, yet remains functionally active against unwanted, pathological oxidative attack. The deuterium presents low risk (see below) in that it is stably incorporated in covalent bonds, and the amount dosed is relatively low. Thus, the risk-benefit profile is thought to be favorable and suggests a future potential benefit of RT001 treatment in this intractable condition.

In models of neurodegenerative diseases, possible triggers such as an aberrant protein (e.g., beta amyloid or synuclein), the low expression of frataxin in FA, chemical or environmental toxins, or redox imbalances, induce a stress in the cell that is first manifested in lipid peroxidation inside mitochondria where several factors combine to make the cells most susceptible. Those factors include: 1) the high concentration of ROS generated by cellular energy generation; 2) the concentrated accumulation of highly susceptible PUFAs in the mitochondrial inner membrane; and 3) inadequate protection by antioxidants due to the hydrophobic nature of the inner membrane that limits antioxidant solubility and diffusion into the susceptible domains. Most important to our mechanism of action is the recognition that the accelerated free radical chain reaction that results from disease amplifies damage massively through an autocatalytic process that propagates recurring damage from a single initiating event. The initiating event is a slow, rate determining step that is self-propagating in its damage to membranes. Stabilizing the bonds most susceptible to oxidation in the initiating event can shut down an entire cascade of accelerating damage in a non-stoichiometric fashion and can provide a new treatment modality for disorders of lipid peroxidation.

RT001 is a site-specific deuterated analogue of LA ethyl ester. LA is designated by the FDA as GRAS with no upper limit set by toxicity. The exposure to LA in the typical Western diet ranges from 5 to 20 g/day. Intralipid® 20%, a product approved by FDA for intravenous (IV) parenteral nutrition, can be administered at doses which provide up to 93 g/day of LA (2.5 g/kg of Intralipid which is up to 2/3 LA). As demonstrated in nonclinical and *in vitro* studies, there were no

demonstrable adverse metabolic effects of deuterating LA. Likewise, the amount of deuterium to be administered at the highest dose proposed for use in this clinical study is approximately four-orders of magnitude lower than the safety threshold described by the International Atomic Energy Agency (IAEA, 2009).

Linoleic acid is the predominant fatty acid in two emulsions approved by the FDA as a source of calories and essential fatty acids. Intralipid 10% (Cutter Pharmaceuticals, IND 17-643) was submitted as an NDA on August 9, 1974. Also, around this time, Liposyn 10% (Abbott Laboratories, IND 18-203) also was filed. These intravenous formulations were developed to provide nutrition to patients who had conditions which precluded effective enteral feeding. Subsequent to these approvals, some changes have been made to the concentration and plant source for the various products, but the component fatty acids have remained consistent.

Intralipid is predominantly soybean oil in concentrations of 10%, 20% and 30%. Egg yolk phospholipids are added in a concentration of about 1.2%. Liposyn includes both soybean and safflower oil in the identical concentrations. The approximate major component fatty acids include linoleic (65%), oleic (20%), palmitic (5%), linolenic (5%), and stearic (5%). Intralipid was approved based on the results of 67 studies conducted before approval. These included 4 studies in 22 human volunteers, and 63 studies in 298 patients. Of the patients included in the studies, 128 were adults, and 170 were pediatric patients (mostly infants). The studies showed that the product was pharmacologically inactive and relatively well-tolerated. Most adverse drug reactions were related to the route of administration rather than to the systemic effects of the product itself. These reactions included: local vein reaction, sepsis, allergic reactions, hyperlipidemia, fever, sleepiness, headache, nausea, vomiting, flushing, hypercoagulability, thrombophlebitis, thrombocytopenia, leukopenia, tachycardia, dyspnea, splenomegaly, and shock.

Post approval, deaths were reported in association with Intralipid use in pre-term infants. This was thought due to fat accumulation in the lungs in these infants. The maximum dose used in pediatric patients was reduced to 3 g/kg/day, and a warning placed on the label for use in pediatric patients.

Deuterated LA and other deuterated PUFAs have been used safely and extensively in biochemistry and nutrition research for several decades to elucidate the metabolic pathways for fatty acid metabolism, cell membrane integrity and prostaglandin synthesis (Brenna, 1997). Single and multiple doses of deuterated PUFAs (up to 10-18 g) have been used in clinical studies without adverse reactions. A wide variety of deuteration positions on LA have been used for those studies, and some, including perdeuterated LA, included deuterium at the C11 position. For example, Emken and colleagues investigated the desaturation of 18:0 and 16:0 and 18:2n-6 and 18:3n-3 elongation/desaturation in four healthy adults, who received oral doses of about 3-3.5 g of $d_2$-18:2n-6, $d_4$,-18:3n-3, and $d_6$-18:n-9 fatty acids (Emken et al., 1990). Salem and colleagues

have safely administered $d_5$-18:2n-6 and $d_5$-18:3n-3 doses of 50-10 mg/kg body weight to premature infants and showed that infants as small as 1,980 g and 32 weeks gestation elongate and desaturate both precursors within 24 hours (Salem et al., 1996).

Based on the initial analysis of data from RT001-002, RT001 remains very safe. The maximal tolerated adult dose studied is approximately 9.0 g/d above which steatorrhea/diarrhea may occur. In the initial 28-day study, signs of clinical activity may have been demonstrated. These changes in clinical activity markers were not expected in this 28-day study, but they form the basis for Study RT001-006 where RT001 will be dosed for 11 months.

## 6.9. Description and Justification for the Dosing Regimens to Be Studied

LA cannot be synthesized by mammals and is an essential component of the human diet. LA is the most common dietary source of polyunsaturated fat for humans and is a source of metabolic energy (9 Calories/g) and longer chain omega-6 fatty acids. LA is also the starting compound for other desaturated and elongated fatty acids that are normal physiological lipid components (e.g., arachidonic acid 20:4n-6), an important fatty acid in membrane phospholipids. Nonclinical studies have shown that RT001 recapitulates LA biochemistry in normal physiological lipid processing.

The amount of deuterium contained in the high dose proposed for the clinical study is below doses commonly used for isotope labeling studies. The dose of RT001 will contribute approximately 80 Calories and is within the range of daily fat intake recommended by the Centers for Disease Control (CDC).

RT001 has been administered in humans at a higher level than the current study proposed dosing regimen and was found to be safe in Study RT001-002. There was one treatment emergent adverse event report characterized as serious – steatorrhea associated with the use of study drug. The event resolved after 6 hours. The subject was hospitalized for overnight observation. The subject had no other symptoms and did not receive specific treatment. Upon questioning, a few other subjects in the high dose cohort also reported occasional diarrhea that was mild and not worrisome. The dose for this study is 8.64 g/d for 30 days followed by 5.76 g/day, which is well below the highest tested dose of 9.0 g/day in Study RT001-002.

# 7. STUDY OBJECTIVES

This study is designed to evaluate the effect of RT001 in subjects with FRDA:

## 7.1. Primary endpoint

1. To demonstrate the efficacy of RT001 compared to placebo on the change of MVO2 in patients with FA through change from baseline to 11 months

## 7.2. Key secondary endpoint

1. To demonstrate the efficacy of RT001 compared to placebo on an overall performance score based on a GST change from baseline to 11 months. The GST will be a combination of three endpoints (timed 1 minute walk test [T1MW], peak workload, and MVO2).

## 7.3. Secondary endpoints

1. To demonstrate the efficacy of RT001 compared to placebo on T1MW with change from baseline to 11 months
2. To demonstrate the efficacy of RT001 compared to placebo on the change of peak workload measured by CPET through change from baseline to 11 months

## 7.4. Exploratory endpoints

To demonstrate the efficacy of RT001 on change from baseline at 11 months in clinical symptoms as measured by:

1. Modified Friedreich's Ataxia Scale (mFARS)
2. Fatigue Symptom Scale (items 2. 4. 7.9 of the total fatigue scale)
3. Clinician-rated Clinical Global Impression of Change (CGI-C)
4. Activities of daily living (ADL) elements of fatigue scale (items 5,6, 8, 10, and 11)
5. Patient Global Impression of Change (PGI-C)

- To evaluate the effects of RT001 on Visual Analogue Scale [VAS]
- To evaluate the effects of RT001 on speech measurements and Speech Assessment questionnaire
- To evaluate the effect of RT001 on T25FW
- To evaluate the effects of RT001 on FARS-Neuro
- To evaluate the effects of RT001 on FARS-ADL

- To evaluate the effects of RT001 on SF-36
- To evaluate the effects of RT001 on the Neuro-QoL scales (Upper Extremity Function and Lower Extremity Function)
- To evaluate the effects of RT001 on the frequency of falls as reported in a fall diary and subject's fear of falling
- To evaluate the effects of RT001 on the Patient Global Impression of Change (PGI-C) Scale
- To evaluate the effects of RT001 on efficacy assessments after 4 months of treatment
- To evaluate the effects of RT001 on efficacy assessments after 8 months of treatment
- To evaluate the effects of RT001 on ejection fraction as reported in the Echocardiogram
- To evaluate the effects of RT001 on hypertrophy as reported in the Echocardiogram

## 7.5. Optional Sub-Study Objectives

### 7.5.1. Optional PK Profile Sub-Study

The objective of the study is to evaluate the pharmacokinetic (PK) profile of RT001 at steady state.

### 7.5.2. Optional PK Washout Sub-Study

The objective of the study is to evaluate the washout blood levels of RT001 after treatment in RT001-006 is completed.

# 8. INVESTIGATIONAL PLAN

## 8.1. Study Design

This is a randomized, double-blind, placebo-controlled study to evaluate the efficacy, safety, and tolerability in subjects with FRDA following the oral administration of study drug (active or placebo capsules).

Subjects will self-administer the study drug orally with meals. While on study drug, subjects are recommended to avoid eating foods high in PUFAs. Subjects will keep food journals from screening through the completion of month 1 on study drug. After month 1, subjects will keep 2-week food journals prior to next scheduled onsite visit. Subjects (or parents/caregivers, as applicable) will have an initial call and as needed with the diet coach to discuss food journal completion requirements.

A total 60 FRDA subjects will be enrolled. Subjects will be randomized to active [9 capsules of RT001 (8.64 g/day) for the first month, followed by 6 capsules of RT001 (5.76 g/day)] or placebo in a 1:1 ratio. Randomization will be stratified by FARS Neurological score: ≤45 and >45. Enrollment of patients with age > 25 will be capped at 25 % of the total enrollment.

Subjects will self-administer orally 9 capsules daily for the first month with a meal: 3 capsules with breakfast, 3 capsules with lunch, and 3 capsules with dinner for the first month. After this initial period the dose will be reduced to 6 capsules daily with a meal: 3 capsules with breakfast, and 3 capsules with dinner for the remainder of the trial, while on study drug. If a subject is unable to tolerate study drug because of adverse events during the initial loading period, the dose may be reduced to six capsules daily (5.76 g total dose) given as 3 capsules with breakfast, and 3 capsules with dinner. If a subject is unable to tolerate study drug because of adverse events after the dose reduction to six capsules daily (5.76 g total dose), the dose may be spread over three meals (2 capsules at breakfast, 2 capsules with lunch, and 2 capsules with dinner). If the subject is still unable to tolerate study drug after spreading it over three meals, the total dose may be reduced by 1-2 capsules/day, preferably by reducing the dose for a meal from 2 to 1 capsule(s) as needed. After 2 weeks of taking a dose of less than 6 caps/day, the subject should try to increase the dose to 6 capsules/day given as 2 capsules TID given with meals. If 6 caps/day is not tolerable, the subject can take the drug at the dose of less than 6 caps/day that was tolerable. The last dose of study drug will be taken at 4 weeks from Day 330 visit.

Participation is anticipated to require 13 months.

- **Screening**: Up to 30 days from start of treatment
- **Treatment Period:** treatment for a 12-month period

Safety assessments include physical and neurologic examinations, vital signs, 12-lead electrocardiogram (ECG) tracing, and clinical laboratory tests (hematology, clinical chemistry,

lipid profile, coagulation, and urinalysis) to identify adverse events (AEs). Adverse events will be evaluated for incidence, severity, and relationship to study drug.

The first dose of the study drug will be taken on Day 1. Bioanalytical measurements will be performed to determine the concentration of D2-LA, deuterated arachidonic acid (D2-ARA), nondeuterated linoleic acid (H2-LA) and nondeuterated arachidonic acid (H2-ARA) in plasma and red blood cells. Blood samples will be collected at month 4 (Day 120 ± 30 days), at month 8 (Day 240 ± 30 days), and at month 11 (Day 330 ± 30 days). All samples are to be obtained prior to dosing and prior to breakfast.

Subjects will perform CPET on a stationary recumbent bike at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Peak work rate and $VO_2$ MAX will be measured during incremental exercise testing. CPET should occur after fasting (if the subject has had a meal/snack) for a minimum of 2 hours, with neither caffeine nor nicotine for at least 12 hours before testing.

Subjects (with or without assistive device) will perform a T1MW and electronically measured T25FW at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days).

CPET and the T1MW/T25FW should be separated in time to allow the subject to recover sufficiently before doing the next test. All tests should be done at approximately the same time and order for each visit.

The FARS-Neuro and clinician-rated CGI scale will be performed at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days) by a qualified physician or health professional who is trained on the use of the tool. The CPET examination should always precede the FARS-Neuro evaluation at all visits.

A trained health professional will also work with the subject to perform the speech measurement recording at Screening, on Day 1, Day 240 (± 30 days), and Day 330 (± 30 days).

The FARS-ADL, SF-36, VAS (for Fatigue), Fatigue scale, Neuro-QoL scales (lower extremity function and upper extremity function) and Fall Questionnaire will be completed by the subjects at screening and at the visits on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). The PGI-C scale and VAS (for Improvement of FA symptoms) will be completed by all subjects at Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days).

In between the clinic visits, subjects will also be contacted by phone by a study site staff member and asked questions relating to the PGI-C, Fatigue scale, Neuro-QoL Scales (lower extremity function and upper extremity function), and questions 8 and 9 of the fall questionnaire. These phone 'visits' will take place monthly. A Speech Assessment Questionnaire will be completed by the subject and parents/caregiver (as applicable) at Screening, on Day 1, Day 240 (± 30 days), and Day 330 (± 30 days).

Between clinic visits, subjects will be asked to keep a daily log (diary) documenting falls, near-falls, stumbles, and fear of falling (Screening through Day 330).

Additional blood samples will be collected and stored for future biomarker analysis on Day 1, on Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). These samples are to be obtained prior to dosing and prior to breakfast.

A final phone call will occur 4 weeks (+ 7 days) from the Day 330 visit to confirm date of last dose and accountability of final bottles. The site will also collect AE information and confirm any change in concomitant medication since the last visit. Any ongoing AEs at EOT will be followed to conclusion or until stabilized.

Two additional optional sub-studies will be offered. The first is a 24hr PK Profile at steady state that will occur before or after the Day 330 visit. Immediately before or after the Day 330 visit in RT001-006, PK samples will be obtained 30 minutes to 1 hour before the morning dose; and 30 min, 1, 2, 4, 8, 12 and 24 hours after the morning dose.

The second optional sub-study is a 6-month study to evaluate the washout blood levels of RT001 after treatment in RT001-006 is completed. Depending on the timing of entry into the sub-study, washout pharmacokinetic (PK) samples will be obtained at 7 days, 4, 12, and 24 weeks after the last dose to determine the trough concentration of deuterated linoleic acid (D2-LA), deuterated arachidonic acid (D2-ARA), nondeuterated linoleic acid (H2-LA) and nondeuterated arachidonic acid (H2-ARA) in plasma, and D2-LA, D2-ARA H2-LA, and H2-ARA in red blood cells.

## 8.2. Randomization

The randomized, double-blind design of the study is intended to minimize bias on the part of subjects, investigators, and analysts. Subjects who meet all enrollment criteria and from whom consent has been obtained will be randomly assigned to receive one of two treatments (RT001 or placebo capsules) following a 1:1 ratio. Randomization will be stratified as much as possible according to the FARS Neurological score (baseline FARS score of ≤45 and >45). Enrollment of patients with age > 25 will be capped at 25 % of the total enrollment.

## 8.3. Blinding

Subjects and study personnel, including but not limited to investigators, study coordinators, nursing staff, clinical monitors, the Sponsor, and the Sponsor's representatives, will remain blinded to the study treatment assignments (RT001 or placebo).

If there is a need to unblind the study drug assignment for a subject, the Principal Investigator will discuss with the sponsor's Medical Monitor. A separate unblinding plan will document the specific processes related to emergency unblinding. Refer to Section 15.7 for a description of emergency unblinding procedures.

The reason for unblinding must be documented in the source document for the subject. If a subject's study drug assignment is unblinded, the subject should remain in the study and continue the protocol-specified follow-up evaluations.

## 8.4. Safety Monitoring

### 8.4.1. Safety Monitoring

The study site investigators assess each subject and record findings obtained during each visit. Subjects will be queried to report any observations, concerns or symptomatology, and a clinical assessment will also be made. This includes any evidence of disease exacerbation and/or progression. Safety reports made during the study (see Section 15) will be promptly reviewed by the Sponsor's Medical Monitor and processed. This includes reporting to the FDA according to 21 CFR 312.32, "IND Safety Reporting," and taking any action necessary to protect subject safety.

### 8.4.2. Sponsor Safety Monitoring

The blinded safety and efficacy data will be reviewed by the study monitor on an ongoing basis. This data will not be made available to the Principal Investigators unless changes need to be made to the protocol for safety reasons.

### 8.4.3. Data Safety and Monitoring Board

A Data Safety and Monitoring Board (DSMB) will be established to review this study and provide recommendations per the DSMB Charter.

# 9. SELECTION AND WITHDRAWAL OF SUBJECTS

## 9.1. Inclusion Criteria

To be enrolled into the study subjects must meet the following requirements:

1. Male or female 12 to 50 years of age
2. Medical history consistent with the symptoms of FRDA at ≤ 25 years of age
3. Detection of biallelic pathogenic variants in frataxin gene (FXN) (i.e. biallelic repeat expansions of GAA in the disease-causing range, or compound heterozygous for a repeat expansion of GAA in the disease-causing range + an intragenic pathogenic variant, etc.)
4. Ambulatory (with or without assistive device) and capable of performing other assessments/evaluations
5. Must be able to walk 25 feet during the timed 1-minute walk
6. Agree to receive calls from a diet coach regarding completion of electronic food journal
7. Sign the informed consent form prior to entry into the study
8. Agree to stay locally for the required visit days
9. Able to provide the necessary repeated blood samples

### 9.1.1. Inclusion Criteria for Optional PK Profile Sub-Study

1. At selected sites, subjects who are enrolled in RT001-006 will be invited to participate in this study.
2. All participating subjects will be required to sign an informed consent prior to the collection of the blood draws.
3. Subjects must be willing to stay overnight close to the study site and able to provide the necessary repeated blood samples.

### 9.1.2. Inclusion Criteria for Optional PK Washout Sub-Study

1. Subjects who have successfully completed treatment in RT001-006 will be invited to participate in the washout PK sub-study.
2. All participating subjects will be required to sign an informed consent prior to any blood draws.
3. Subjects must be willing and able to provide the necessary repeated blood samples.

## 9.2. Exclusion Criteria

Subjects meeting one or more of the following may not enter the study:

1. Received treatment with other experimental therapies within the last 30 days prior to the first dose
2. Previously participated in the RT001 trial
3. Refusal to discontinue fish oils or other oil-based supplements for the duration of the study (Screening till last study procedure completed)
4. History of malignancies (other than basal cell carcinomas)
5. Inability to complete CPET protocol, as, for example, unable to move the pedals on the exercise bicycle, inability to maintain sufficient RPMs to finish the study, or as judged by the exercise physiologist supervising the testing
6. Female who is breastfeeding or has a positive pregnancy test
7. Male participant or female participant of childbearing potential, who is sexually active and unwilling/unable to use a medically acceptable and effective double barrier birth control method throughout the study
8. Unwilling or unable to comply with the requirements of this protocol, including the presence of any condition (physical, mental, or social) that is likely to affect the subject's ability to return for visits as scheduled
9. Clinically significant cardiac abnormalities (e.g., left ventricular ejection fraction [LVEF] <40% on a recent echocardiogram [obtained within 12 months prior to screening] or clinically unstable arrhythmia) at screening that, in the opinion of the Investigator, would make the subject unsuitable for enrollment
10. History of or currently uncontrolled diabetes mellitus (Type 1 or 2)
11. Suicidal ideation within the last 12 months as determined by the Columbia-Suicide Severity Rating Scale (C-SSRS)
12. History, within the last 2 years, of alcohol abuse, significant mental illness, or physical opioid dependence

### 9.2.1. Exclusion Criteria for Sub-Studies

There are no exclusion criteria for the two additional sub-studies.

## 9.3. Withdrawal

### 9.3.1. Withdrawal Criteria

Subjects may choose to withdraw from the study at any time for any reason. In addition, subjects may be withdrawn from the study by the Investigator for any of the following reasons:

- The subject is unwilling or unable to adhere to the protocol.

- During the course of the study, the subject develops symptoms or conditions listed in the exclusion criteria.
- Any SAE, clinically significant AE, severe laboratory abnormality, intercurrent illness, or other medical condition that indicates to the Investigator that continued participation is not in the best interest of the subject.
- Other medical reason, at the discretion of the Investigator and/or the Medical Monitor.

### 9.3.2. Withdrawal Procedures

- If it is necessary for a subject to discontinue the study drug/study earlier than planned, subjects should complete Early Termination procedures (see Section 13.13) and return the study drug, as applicable. Date of last dose should be recorded.
- The Investigator must notify the Sponsor and the Medical Monitor within 24 hours when a subject has been withdrawn from the study. Any subject withdrawn because of a related AE (whether serious or non-serious), including clinically significant abnormal laboratory test values, will be evaluated by the Investigator or a designee and be treated and/or followed until the symptoms resolve or values return to normal or acceptable levels, as judged by the Investigator.
- If a subject does not return for a scheduled visit, every effort should be made to contact the subject. If a subject withdraws from the study, the Investigator or designee should inquire about the reason for withdrawal, and follow-up with the subject by phone as scheduled to collect AE information.
- If the subject withdraws from the study and also withdraws consent for disclosure of future information, no further evaluations will be performed, and no additional data will be collected. The Sponsor may retain and continue to use any data collected before such withdrawal of consent; these data will be included in the safety database.

### 9.3.3. Documentation of Withdrawal

The reason(s) for withdrawal from the study drug/study must be recorded in the subject's medical record and case report forms (CRF).

### 9.3.4. Replacement of Subjects

Subjects who discontinue before enrollment is complete may be replaced in the study. Replacement subjects will be assigned unique subject numbers.

### 9.3.5. Termination of Study by Sponsor

Although the Sponsor has every intention of completing the study, the Sponsor reserves the right to discontinue the study at any time for clinical or administrative reasons, or if required by the

FDA or other regulatory authorities. Both the Sponsor and the Medical Monitor will review the safety of RT001 throughout the study. The study may be halted at any time for safety concerns.

## 9.4. Pregnancy

Pregnant women are not eligible for inclusion in the study. Before enrolling women of childbearing potential (WOCBP) in this clinical trial, Investigators must advise WOCBP of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. WOCBP must agree to use a medically acceptable and effective barrier method of contraception (e.g., condom with spermicide, birth control pill) prior to study entry and for the duration of study participation to avoid pregnancy throughout the study and for a period of at least 30 days after all study procedures are completed. Male subjects must also agree to use an accepted method of contraception for the duration of the study and for a period of at least 30 days after all study procedures are completed. This should be covered during the informed consent process.

# 10. INVESTIGATIONAL PRODUCT

## 10.1. Identity of Investigational Product

The investigational product is RT001. RT001 is encapsulated 9-*cis*, 12-*cis*-11,11-$D_2$-linoleic acid ethyl ester, which is a site-specific (C11) di-deutero synthetic homologue of LA ethyl ester. Each capsule contains 960 mg of RT001. The placebo product is composed of encapsulated USP safflower oil. The placebo capsules are identical in appearance and size to the investigational drug product. The study drug and placebo are indistinguishable. Both are described as opaque brown oblong soft-gel capsules.

## 10.2. Packaging and Labeling of Clinical Supplies

RT001 and placebo are supplied as soft-gelatin (softgel) capsules. RT001 and placebo capsules are provided in identical glass bottles each containing 42 softgel capsules.

## 10.3. Storage of Clinical Supplies

Study drug should be stored by the study site in a controlled refrigerated temperature of 2 to 8°C (35.6 to 46.4 °F). If the temperature exceeds or falls below this range, please report it to the Sponsor as outlined in the Pharmacy Manual. Study drug should be refrigerated by the subject once dispensed from the site. Please refer to Pharmacy manual for further details.

## 10.4. Study Drug Dispensation

The study drug for the first 4 weeks of dosing will be dispensed at the study sites. Study drug for the subsequent dosing may be shipped directly to the subject or site from a drug depot or site, depending on the dispensing schedule.

## 10.5. Drug Accountability

Subjects will be asked to return the used and unused study bottles to the study site or send it back to the depot. It is the responsibility of the Investigator or his/her designee to maintain drug accountability at the clinical trial site and ensure that a current record of investigational product disposition is maintained. It is the responsibility of the Investigator or his/her designee to ensure that the investigational product is used only in accordance with the approved protocol. All records or logs must comply with applicable regulations and guidelines. The Sponsor will provide forms to facilitate accountability if the staff at the investigational site does not have an established system that meets these requirements.

# 11. STUDY DRUGS AND OTHER MEDICATIONS, SUPPLEMENTS

## 11.1. Study Drug Administration

Subjects will receive instructions on taking the study drug with meals. RT001 should be administered with food, preferably at mealtime. On clinic visit days all subjects will receive the morning dose of study drug in the clinic.

Subjects will self-administer orally 9 capsules daily for the first month with a meal: 3 capsules with breakfast, 3 capsules with lunch, and 3 capsules with dinner for the first month. After this initial period the dose will be reduced to 6 capsules daily with a meal: 3 capsules with breakfast, and 3 capsules with dinner for the remainder of the trial, while on study drug. If a subject is unable to tolerate study drug because of adverse events during the initial loading period, the dose may be reduced to six capsules daily (5.76 g total dose) given as 3 capsules with breakfast, and 3 capsules with dinner. If a subject is unable to tolerate study drug because of adverse events after the dose reduction to six capsules daily (5.76 g total dose), the dose may be spread over three meals (2 capsules at breakfast, 2 capsules with lunch, and 2 capsules with dinner). If the subject is still unable to tolerate study drug after spreading it over three meals, the total dose may be reduced by 1-2 capsules/day, preferably by reducing the dose for a meal from 2 to 1 capsule(s) as needed. After 2 weeks of taking a dose of less than 6 caps/day, the subject should try to increase the dose again to 6 capsules/day given as 2 capsules TID given with meals. If 6 caps/day is not tolerable, the subject can take the drug at the dose of less than 6 caps/day that was tolerable. The last dose of study drug (3 capsules) will be taken 4 weeks from the Day 330 visit.

## 11.2. Prior and Concomitant Medications

Stable, ongoing therapies will be permitted during this study. Any drug therapy initiated during the study should be discussed with the Medical Monitor prior to administration, if possible. Data on concomitant medications will be collected at each visit.

Fish oils or other oil-based supplements should not be taken for the duration of the study. In addition, if subjects have participated in prior therapeutic trials, they will need to have been off therapy for at least 30 days prior to receiving the first dose of study drug. Changes in herbal remedies and changes in prescription medications are strongly discouraged during the trial.

## 11.3. Subject Compliance

Compliance will be assessed through counting the number of bottles/capsules returned to the investigational site by the subject. The site will contact the subject to confirm date of last study dose as well as to perform final drug accountability during the EOT call. The subject will be given instructions on how to return any unused study drug/bottles to the site.

# 12. EFFICACY ENDPOINTS, SAFETY, PHARMACOKINETIC, AND OTHER ASSESSMENTS

## 12.1. Efficacy Endpoints

Efficacy endpoints include the following:

- Exercise testing using CPET (peak workload and $VO_2MAX$)
- T1MW and T25FW
- Speech measurement recording
- Clinician-rated Scales
    - FARS-Neuro
    - Clinician-rated CGI
- Questionnaires and scales completed by the subject
    - FARS-ADL
    - SF-36
    - Patient Global Impression of Change (PGI-C)
    - VAS
    - Fatigue scale
    - Neuro-QoL scale (lower extremity function and upper extremity function)
    - Fall Questionnaire
    - Speech Assessment Questionnaire
- Frequency of falls, near falls, and stumbles as reported in a fall diary

## 12.2. Exercise Testing Using CPET

Subjects will perform exercise testing using the CPET protocol at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Peak work rate and $VO_2$ MAX will be measured during incremental exercise testing on a recumbent bike. All tests should be done at approximately the same time for each visit. CPET should occur after fasting (if the subject has had a meal/snack) for a minimum of 2 hours, with neither caffeine nor nicotine for at least 12 hours before testing. Fluid consumption during the fasting period is limited to water, preferably at room temperature. Physical activity during the 24 hours prior to testing should be minimized. Routines followed for the initial laboratory assessments with respect to diet and physical activity should be duplicated for each subsequent visit. Subjects may take as much resting time as they

need between the bike test and the T1MW/T25FW. Detailed testing instructions will be provided in a separate manual.

## 12.3. Timed Walk

### 12.3.1. Timed 1-Minute Walk (T1MW)

The T1MW is a quantitative measure of lower extremity function. The subject is instructed to walk for 1 minute as quickly and safely as possible on a clearly marked course. The distance walked over 1 minute is measured. The T1MW will be performed on subjects who are ambulatory (with or without assistive device). This will be evaluated at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days).

### 12.3.2 Timed 25-Feet Walk (T25FW)

The T25FW is a quantitative measure of lower extremity function. The subject is instructed to walk 25 feet as quickly and safely as possible. The time to walk 25 feet will be measured electronically. The T25FW will be performed on subjects who are ambulatory (with or without assistive device). This will be evaluated at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Detailed testing instructions will be provided in a separate manual.

## 12.4. Speech Measurement Recording

The subject will have to undergo a speech recording for about 10 minutes at Screening, on Day 1, Day 240 (± 30 days), and Day 330 (± 30 days). This recording will include (but not limited to) (1) reading a brief passage, (2) responding to a task: e.g. days of the week (3) syllable repetition: e.g. saying PATAKA as quickly and as clearly as possible; (e) monologue: on a topic of choice. Details will be included in a separate manual.

## 12.5. Clinician-rated Scales

### 12.5.1. FARS-Neuro

The FARS-Neuro scale includes evaluations of the neurological signs that specifically reflect neural substrates affected in subjects with FRDA. Based on a neurological examination, bulbar, upper limb coordination, lower limb coordination, peripheral nervous system, and upright stability functions are assessed for individual sub-scores (11, 36, 16, 26, and 36) with a maximum score of 125 (Friedreich's Ataxia Study Group, Subramony et al., 2005, Lynch et al., 2006). FARS-Neurologic examinations will be conducted by a qualified physician or health professional listed on the Form FDA 1572 who is trained on use of the FARS-Neurologic scale format. This scale will be evaluated at Screening, on Day 1, Day 120 (± 30 days), Day 240

(± 30 days), and Day 330 (± 30 days). The CPET examination should always precede the FARS-Neuro evaluation at all visits. Refer to Section 19.2 for the specific items assessed.

### 12.5.2. Clinician-rated Clinical Global Impression (CGI)

The CGI is a 3-item observer-rated scale that measures illness severity, global improvement and therapeutic response. The illness severity is rated on a 7-point scale, with the severity of illness scale using a range of responses from 1 (normal) through 7 (amongst the most severely ill patients). The global improvement scores range from 1 (very much improved) through to 7 (very much worse). Treatment response ratings should take account of both therapeutic efficacy and treatment-related adverse events and range from 0 (marked improvement and no side-effects) and 4 (unchanged or worse and side-effects outweigh the therapeutic effects). Each component of the CGI is rated separately; the instrument does not yield a global score. This scale will be evaluated at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). At screening and Day 1, only the severity of illness (question 1) needs to be assessed. From Day 120 onwards, all 3 items (severity of illness, global improvement, and therapeutic response) will be assessed. Refer to Section 19.3 for the specific items assessed.

## 12.6. Questionnaires and scales completed by the subject

### 12.6.1. FARS -Activities of Daily Living (FARS-ADL)

The subject self-administered FARS-ADL is a sub-scale of the FARS. There are nine questions regarding activities of daily living. The ADL is scored from 0 to 36, with higher score representing more severe progression. The FARS-ADL will be collected at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Refer to Section 19.4 for the specific items assessed.

### 12.6.2. SF-36

The SF-36 is a well validated 36-item subject self-administered Short Form Health Survey and is a set of generic, coherent and easily administered quality-of-life measures. The SF-36 will be collected at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Refer to Section 19.5 for the specific items assessed.

### 12.6.3. Patient Global Impression of Change (PGI-C) Scale

The Patient Global Impression of Change (PGI-C) Scale is a qualitative assessment of change completed by the subject using a 7-item scale ranging from "very much worse" to "very much improved". The PGI-C will be collected at Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). In between the clinic visits the subject will be contacted by phone by a study site staff member and asked to complete the PGI-C. This will be done after 30, 60, 90, 150,

180, 210, 270 and 300 days of dosing. All calls have a window of ± 7 days. Refer to Section 19.6 for the specific items assessed.

### 12.6.4. Visual Analogue Scale (VAS)

The subject will complete a VAS for Improvement of FA Symptoms and a VAS for fatigue. The VAS for Fatigue will be completed at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). VAS for Improvement of FA Symptoms will be completed on Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Refer to Section 19.7 for the VAS.

### 12.6.5. Fatigue Scale

The subject will complete a Fatigue scale at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). In between the clinic visits the subject will be contacted by phone by a study site staff member and asked to complete the Fatigue scale. This will be done after 30, 60, 90, 150, 180, 210, 270 and 300 days of dosing. All calls have a window of ± 7 days. Refer to Section 19.8 for the specific items assessed.

### 12.6.6. Neuro-QoL scales

The subject will complete the Neuro-QoL scale for upper extremity function and lower extremity function. These scales will be collected at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). In between the clinic visits the subject will be contacted by phone by a study site staff member and asked to complete the Neuro-QoL scales. This will be done after 30, 60, 90, 150, 180, 210, 270 and 300 days of dosing. All calls have a window of ± 7 days. Refer to Section 19.9 for the specific items assessed.

### 12.6.7. Fall Questionnaire

The subject will complete a Fall questionnaire at Screening, on Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). In between the clinic visits the subject will be contacted by phone by a study site staff member and asked to complete questions 8 and 9 of the fall questionnaire. This will be done after 30, 60, 90, 150, 180, 210, 270 and 300 days of dosing. All calls have a window of ± 7 days. Refer to Section 19.10 for the specific items assessed.

### 12.6.8. Fall Diary

The subjects will be asked to keep a daily log (diary) documenting falls, near-falls, stumbles, and fear of falling from screening until the end of treatment. Falls are defined as any event that results in unintentionally falling to the ground. Near-falls are defined as losing balance with no fall to the ground (e.g. hold on to something/somebody, or fall onto a chair, bed, or other soft landing). Stumbles are defined as losing balance momentarily with regaining of footing and no fall.

### 12.6.9. Speech Assessment Questionnaire

The subject will complete a Speech Assessment Questionnaire to evaluate his/ her speech pattern and communication style at Screening, on Day 1, Day 240 (± 30 days), and Day 330 (± 30 days). Parent/caregiver/travel companion will also have to complete one question on this questionnaire. Refer to Section 19.11 for the specific items assessed.

## 12.7. Safety

Safety assessments include AEs, physical and neurologic examinations, vital signs, 12-lead electrocardiogram (ECG), and clinical laboratory tests (hematology, clinical chemistry, lipid profile, coagulation, and urinalysis). Safety signals must be promptly communicated to the Medical Monitor.

### 12.7.1. Adverse Events

Subjects will be assessed for AEs at each study visit after the informed consent is obtained through the end of the study. Adverse events will be evaluated for incidence, severity, and relationship to study drug. The safety evaluation will include an assessment of all AEs, SAEs, and dose discontinuations due to adverse event. Monitoring and grading of AEs will be done in accordance with Medical Dictionary for Regulatory Activity (MedDRA) during the study. See Section 15 for additional information.

### 12.7.2. Physical Examination

Complete physical examinations will be conducted at Screening, Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days). Physical examinations include skin, HEENT, respiratory, cardiovascular, gastrointestinal, endocrine/metabolic, neurological, blood/lymphatic, musculoskeletal, hepatic, allergies and psychological/psychiatric examinations.

### 12.7.3. Vital Signs

Vital signs (systolic and diastolic blood pressure, heart rate, respiration rate, temperature) and weight will be measured at each site visit. All measurements should be performed in the supine/semi-supine or seated position after the subject has rested in that position for at least 3 minutes. Blood pressure will be obtained with an automated or manual blood pressure apparatus in the same position. Blood pressure and pulse should be performed on the same extremity throughout the study and documented as such. Height and BMI will be measured at Screening and at Day 330.

### 12.7.4. 12-Lead Electrocardiogram

A single ECG reading will be obtained at each site visit [Screening, Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days)]. If the Screening and Day 1 visit are within

7 days (or less) of each other, the ECG does not have to be repeated, unless to rule out any clinically significant findings. A copy of the tracing should be retained as a source document. The Investigator will review each ECG and provide an interpretation of the ECG as to whether the ECG is: (1) within normal limits compared with Baseline and within normal limits for a FRDA population, (2) or abnormal with any clinically significant findings noted. Clinically significant changes will be recorded as AEs.

Individuals with FRDA show substantial variance in ECG readings; typical ECG changes in subjects with FRDA may be assessed as clinically significant in other populations. Underlying disease must be considered when evaluating ECG changes.

### 12.7.5. Clinical Laboratory Tests

The following clinical laboratory tests will be performed at each site visit [Screening, Day 1, Day 120 (± 30 days), Day 240 (± 30 days), and Day 330 (± 30 days)] at a local approved laboratory. If the Screening and Day 1 visit are within 7 days (or less) of each other, the laboratory tests do not have to be repeated, unless to rule out any clinically significant findings. The safety laboratory tests, and lipid profile should be performed in a fasting state:

- Hematology: hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular volume, mean platelet volume, platelet count, red blood cell (RBC) count, and white blood cell (WBC) count with differential (absolute).
- Coagulation: prothrombin time (PT), international normalized ratio (INR), and partial thromboplastin time (PTT).
- Clinical chemistry: ALT, AST, alkaline phosphatase, total bilirubin, amylase, lipase, calcium, magnesium, glucose, sodium, potassium, carbon dioxide ($CO_2$), chloride, blood urea nitrogen (BUN), and creatinine.
- Urinalysis (only at screening and Day 330): dipstick test and a microscopic examination if clinically indicated.
- Lipid profile: total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides.

Women will be tested for pregnancy by serum pregnancy test at Screening and by a urine pregnancy test at subsequent visits (including EOT), unless there is documented evidence confirming they are not of childbearing potential. Subjects should be counseled to avoid pregnancy for at least 30 days after all study procedures are completed (see Section 9.4). A Urine Pregnancy test is to be performed by the subject at home on the day of the EOT phone call (before the call and after the last dose). Subjects taking the Urine Pregnancy Test will telephonically confirm the result with the site, scan and email/fax a photo of the result to the site.

### 12.7.6. Columbia-Suicide Severity Rating Scale

This scale will be administered at Screening to identify if the subject has suicidal ideation or suicidal behavior.

### 12.7.7. Echocardiogram

Subjects will need to get an echocardiogram done at Screening if there has not been an echocardiogram obtained in the 12 months prior to screening. An echocardiogram obtained up to 12 months prior to Screening can be used for the assessment of the eligibility in the study. A repeat echocardiogram will be obtained on Day 330 (± 30 days).

## 12.8. Pharmacokinetics

### 12.8.1. Sample Collection

Bioanalytical measurements will be performed to determine the concentration of D2-LA, D2-ARA, H2-LA and H2-ARA in plasma and red blood cells. Blood samples will be collected for plasma measurements and assessment in red blood cells at month 4 (Day 120 ± 30 days), at month 8 (Day 240 ± 30 days), and month 11 (Day 330 ± 30 days). Samples are to be obtained prior to dosing and prior to breakfast. Actual collection time points should be recorded and will be used for PK analysis. Best efforts to collect the samples at the planned time should be made.

Protocol-specific instructions will be provided to each site in a Study Lab Manual for the collection, handling, storage, and shipping of PK (plasma and RBC) samples. The date and actual time each sample is collected will be recorded. Date and time of last dose prior to sample collection will also be recorded.

Two additional optional PK sub-studies will be offered. The first is a 24hr PK Profile at steady state that will occur immediately before or after the Day 330 visit. At the time of the Month 11 visit in RT001-006, PK samples will be obtained 30 minutes to 1 hour before the morning dose (pre-breakfast); and 30 min, 1, 2, 4 (pre-lunch), 8 (pre-dinner), 12 (post-dinner) and 24 hours after the initial morning dose.

The second optional sub-study is a 6-month study to evaluate the washout blood levels of RT001 after treatment in RT001-006 is completed. Depending on the timing of entry into the sub-study, washout pharmacokinetic (PK) samples will be obtained at 7 days, 4, 12, and 24 weeks after the last dose to determine the trough concentration of deuterated linoleic acid (D2-LA), deuterated arachidonic acid (D2-ARA), nondeuterated linoleic acid (H2-LA) and nondeuterated arachidonic acid (H2-ARA) in plasma, and D2-LA, D2-ARA H2-LA, and H2-ARA in red blood cells.

## 12.9. Biomarker Assessment

Additional blood samples will be collected and stored for future biomarker analysis on Day 1, on Day 120 (± 30 days), on Day 240 (± 30 days), and on Day 330 (± 30 days). These samples are to be obtained prior to dosing and prior to breakfast.

Protocol-specific instructions will be provided to each site in a Study Lab Manual for the collection, handling, storage, and shipping of samples. The date and actual time each sample is collected will be recorded. Date and time of last dose prior to sample collection will also be recorded.

## 12.10. Other

### 12.10.1. Food Journal Requirement

While on study drug, subjects are recommended to avoid eating foods high in PUFAs. Subjects will keep food journals from screening through the completion of month 1 on study drug. After month 1, subjects will keep 2-week food journals prior to next scheduled onsite visit. Subjects (or parents/caregivers, as applicable) will have an initial call and as needed with the diet coach to discuss food journal completion requirements.

# 13. STUDY PROCEDURES AND SCHEDULE

All study visits, assessments, and procedures involving study subjects will be performed at a Sponsor-approved investigator site, and assessments and procedures will be performed by study personnel with the supervision of an Investigator. All measurements and results obtained as part of the protocol will be recorded in the CRF. The Schedule of Events is summarized in Table 1. It is recommended, but not required that the procedures should be completed in the order that they are listed to avoid causing fatigue to the subject. Each site visit can be done over multiple days as needed to accommodate for scheduling flexibility. Actual start times of all protocol assessments will be recorded.

## 13.1. Visit 1: Screening (Up to 30 Days)

Screening procedures can be performed from 30 to 14 days prior to the first dose on Day 1. A signed and dated ICF (or assent form) will be obtained from the subject as required by the protocol before any screening procedures are conducted. A signed copy of the ICF will be given to the subject. Screening procedures include the following:

- Evaluate eligibility per inclusion/exclusion criteria
- Perform a serum pregnancy test for all female subjects, unless there is documented evidence confirming they are not of childbearing potential
- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain a urinalysis sample (dipstick test and microscopic examination if clinically indicated)
- Perform the exercise bike test (CPET should occur in the morning after fasting for a minimum of 2 hours {if the subject has had a meal/snack}, with neither caffeine nor nicotine for at least 12 hours before testing)
- Perform a FARS-Neuro and clinician-rated CGI (severity of illness question only)
- Record medical history and demographics
- Record the C-SSRS
- Complete the Speech Measurement Recording
- Perform a physical examination
- Record vital sign measurements, height, weight, and BMI
- Subject complete FARS ADL, SF-36, VAS (for Fatigue), Fatigue scale, Neuro-QoL scales, Speech Assessment Questionnaire and Fall questionnaire

- Obtain T1MW and T25FW
- Obtain an echocardiogram (LVEF) (To be performed if there has been no echocardiogram performed in the last 12 months before screening).
- Obtain a 12-lead ECG
- Sites to train subject on completion requirements for the Fall Diary
- Phone call with the diet coach to review food journal completion requirement

## 13.2. Visit 2: Study Day 1

The following procedures will be performed:

Pre-Dose

- Reassess inclusion/exclusion criteria
- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state (does not have to be repeated if Screening visit is ≤ 7 days and there are no clinically significant findings)
- Perform a urine pregnancy test for all female subjects unless there is documented evidence confirming they are not of childbearing potential
- Perform the exercise bike test (CPET should occur in the morning after fasting for a minimum of 2 hours {if the subject has had a meal/snack}, with neither caffeine nor nicotine for at least 12 hours before testing)
- Perform a FARS-Neuro and clinician-rated CGI (severity of illness question only)
- Update medical history and demographics
- Complete the Speech Measurement Recording
- Record vital sign measurements and weight
- Obtain blood samples for biomarkers prior to the morning meal and dosing
- Randomize subject to study treatment group and dispense study drug
- Perform a physical examination
- Subject complete FARS ADL, SF-36, VAS (for Fatigue), Fatigue scale, Neuro-QoL scales, Speech Assessment questionnaire and Fall questionnaire
- Obtain T1MW and T25FW
- Obtain a 12-lead ECG (does not have to be repeated if Screening visit is ≤ 7 days and there are no clinically significant findings

- Collect fall diary

Dosing

Subjects will take, as per the randomization scheme, the first dose of study drug at the clinic with a meal after all baseline testing is complete.

Post-Dose

- Record concomitant medications
- Record AEs

## 13.3. Days 30 (± 7 days), 60 (± 7 days), and 90 (± 7 days)

Subjects will be contacted by phone by a study site staff member and asked to complete the PGI-C, the Fatigue scale, the Neuro-QoL scales (lower extremity function and upper extremity function), and questions 8 and 9 of the Fall questionnaire. Subjects can scan and email questionnaires to the site. The site is to also confirm if the subject is taking study drug as instructed. Adverse events will also be assessed.

## 13.4. Visit 3: Day 120 (± 30 days)

The following procedures will be performed:

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Perform a urine pregnancy test for all female subjects unless there is documented evidence confirming they are not of childbearing potential
- Record vital sign measurements and weight
- Obtain blood samples for PK and biomarkers prior to the morning meal and dosing
- Perform the exercise bike test (CPET should occur in the morning after fasting for a minimum of 2 hours, with neither caffeine nor nicotine for at least 12 hours before testing)
- Perform a FARS-Neuro and clinician-rated CGI
- Perform a physical examination
- Subject complete FARS ADL, SF-36, PGI-C, VAS, Fatigue scale, Neuro-QoL scales and Fall questionnaire
- Obtain T1MW and T25FW
- Obtain a 12-lead ECG

- Collect fall diary
- Perform drug accountability
- Record concomitant medications
- Record AEs

Dosing

- Subjects will take the morning dose of study drug at the clinic with their breakfast.

## 13.5. Day 150 (± 7 days), Day 180 (± 7 days) and Day 210 (± 7 days)

Subjects will be contacted by phone by a study site staff member and asked to complete the PGI-C, the Fatigue scale, the Neuro-QoL scales (lower extremity function and upper extremity function), and questions 8 and 9 of the Fall questionnaire. Subjects can scan and email questionnaires to the site. The site is to also confirm if the subject is taking study drug as instructed. Adverse events will also be assessed.

## 13.6. Visit 4: Day 240 (± 30 days)

The following procedures will be performed:

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Perform a urine pregnancy test for all female subjects unless there is documented evidence confirming they are not of childbearing potential
- Record vital sign measurements and weight
- Obtain blood samples for PK and biomarkers prior to the morning meal and dosing
- Perform the exercise bike test (CPET should occur in the morning after fasting for a minimum of 2 hours, with neither caffeine nor nicotine for at least 12 hours before testing)
- Perform a FARS-Neuro and clinician-rated CGI
- Perform a physical examination
- Complete Speech Measurement recording.
- Subject complete FARS ADL, SF-36, PGI-C, VAS, Fatigue scale, Neuro-QoL scales, Speech Assessment questionnaire and Fall questionnaire
- Obtain T1MW and T25FW
- Obtain a 12-lead ECG

- Collect fall diary
- Perform drug accountability
- Record concomitant medications
- Record AEs

Dosing

- Subjects will take the morning dose of study drug at the clinic with their breakfast.

## 13.7. Day 270 (± 7 days) and Day 300 (± 7 days)

Subjects will be contacted by phone by a study site staff member and asked to complete the PGI-C, the Fatigue scale, the Neuro-QoL scales (lower extremity function and upper extremity function), and questions 8 and 9 of the Fall questionnaire. Subjects can scan and email questionnaires to the site. The site is to also confirm if the subject is taking study drug as instructed. Adverse events will also be assessed.

## 13.8. Visit 5: Day 330 (± 30 Days)

The following procedures will be performed.

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain a urinalysis sample (dipstick test, microscopic examination)
- Perform a urine pregnancy test for all female subjects unless there is documented evidence confirming they are not of childbearing potential. A Urine Pregnancy test kit will be provided to all female subjects (unless there is documented evidence confirming they are not of childbearing potential) to take home for the EOT visit. The site to instruct the subject that Pregnancy test has to be performed by the subject at home on the day of the EOT phone call (before the call and after the last dose)
- Record vital sign measurements, height, weight, and BMI
- Obtain blood samples for PK and biomarkers prior to the morning meal
- Perform the exercise bike test (CPET should occur in the morning after fasting for a minimum of 2 hours, with neither caffeine nor nicotine for at least 12 hours before testing)
- Perform a FARS-Neuro and clinician-rated CGI
- Perform a physical examination

- Subject complete FARS ADL, SF-36, PGI-C, VAS, Fatigue scale, Neuro-QoL scales, Speech Assessment questionnaire and Fall questionnaire
- Obtain T1MW and T25FW
- Obtain a 12-lead ECG
- Obtain an echocardiogram
- Collect fall diary
- Perform drug accountability, if applicable
- Record concomitant medications
- Record AEs

Dosing

- Subjects will take the morning dose of study drug at the clinic with their breakfast. Subjects to be reminded that the last dose of study drug has to be taken 4 weeks from the Day 330 visit.

## 13.9. Optional PK Profile Sub-Study

The following procedures will be performed on Day 1:

- A blood sample for PK will be obtained at hour -1.0 to -0.5 (pre-breakfast, pre-dose),
- Subjects will take the morning dose of study drug at the clinic with their breakfast,
- A blood sample for PK will be obtained hour 0.5 (± 5 min),
- A blood sample for PK will be obtained hour 1 (± 5 min),
- A blood sample for PK will be obtained hour 2 (± 10 min),
- A blood sample for PK will be obtained hour 4 (± 15 min) (pre-lunch),
- A blood sample for PK will be obtained hour 8 (± 15 min) (pre-dinner),
- A blood sample for PK will be obtained hour 12 (± 60 min) (post-dinner)

The following procedures will be performed on Day 2:

- A blood sample for PK will be obtained on Hour 24 (pre-dose, pre-breakfast)

Dosing:

- The subject will only take the morning dose on the PK collection day. They will skip the evening dose.
- The subject should resume regular dosing schedule after morning PK draw on Day 2.

## 13.10. End of Treatment call (4 weeks from Day 330 + 7 days)

Subjects will be contacted by phone by a study site staff member to make sure they have taken their last dose of study drug and to perform final drug accountability. The subject will be provided with instructions, so they can ship or return all unused study drug/ bottles to the site. If none, the subject can destroy the same.

Site to confirm a urine pregnancy test for all female subjects (unless there is documented evidence confirming they are not of childbearing potential) was performed on the day of the EOT call (before the call and after the last dose). The subject to share the result and email a scanned photo of the result for site records. Study site staff member to also collect AE information and confirm any change in concomitant medication since last visit. For any ongoing AEs at EOT, the site will follow up to conclusion or until stabilized.

## 13.11. Optional PK Washout Sub-Study

Depending on the timing of entry into the sub-study, the following procedures will be performed throughout the Optional Washout PK Sub-Study:

- A blood sample for PK will be obtained 7 days (± 1 day) after the last dose.
- A blood sample for PK will be obtained 4 weeks (± 7 day) after the last dose.
- A blood sample for PK will be obtained 12 weeks (± 7 day) after the last dose.
- A blood sample for PK will be obtained 24 weeks (± 7 day) after the last dose.

## 13.12. Unscheduled Visits

Procedures to be performed at any unscheduled visit are at the discretion of the Investigator, but should at least include safety, laboratory, and health examinations necessary for collection of AE information. Any procedure can be repeated at an unscheduled visit as needed.

## 13.13. Early Termination Visit

If the subject withdraws or is discontinued from the study the following safety procedures should be performed:

- Perform a complete physical examination
- Record vital sign measurements and weight
- Obtain a 12-lead ECG
- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile
- Obtain a urinalysis sample (dipstick test, microscopic examination)
- Perform a urine pregnancy test for all female subjects unless there is documented evidence confirming they are not of childbearing potential

- Record AEs
- Record concomitant medications
- Perform drug accountability

# 14. STATISTICS

## 14.1. Statistical Plan

A complete description of analysis populations and statistical methods will be provided in a formal Statistical Analysis Plan that will be finalized prior to unblinding and statistical analysis.

Analysis populations

- All Patients Population: Any participant with any record in the database. This population includes both screening and randomized subjects. Population will be utilized for descriptive patient counts that include screening subjects.
- Intent-to-Treat (ITT) population: All participants randomized to treatment.
- Modified Intent-to-Treat (mITT) Population: All randomized subjects with at least one post-baseline efficacy evaluation will be included in the mITT population and will be analyzed as randomized. The mITT population will be analyzed for efficacy evaluations.
- Per Protocol (PP) Population: For programming purposes, they will be identified as having no major protocol violations as identified separately by the study team and the Sponsor prior to database lock. Analyses of the PP population will be analyzed as treated.
- Safety population: All participants who receive at least 1 dose of drug or placebo.

Demographic data will be presented using descriptive statistics (e.g., mean, standard deviation, median, and range). Exposure to study drug and reasons for discontinuation will be tabulated. Safety analyses will use the Safety population. Safety variables will be tabulated and presented for all subjects who receive study drug or placebo capsules. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs will be presented by system organ class and preferred term. Adverse events will be presented by severity and relationship to study drug. Changes from Baseline in clinical laboratory parameters and vital signs will be summarized across time. Shift tables will be provided for selected laboratory parameters. Physical examination results will be presented in listings.

Primary, secondary and exploratory efficacy analyses of the 11-month on-treatment period will use the mITT population and the per-protocol population unless otherwise specified.

Change from baseline (CFB) will be analyzed by comparing the change between treatment groups using a mixed model with repeated measures (MMRM). The MMRM will compare the estimated change from baseline between treatments for the primary endpoint. This analysis will assess whether there is a difference in estimated CFB between active and placebo groups at 11 months.

The primary analysis will use the ITT methodology and an MMRM methodology with a random slope and intercept for each individual. Due to the small sample size the model will be optimized by implementation of two risk scores (baseline and slope) that account for multiple baseline covariates in one variable. The risk scores will be derived from blinded data using the GST change from baseline values and generated using the baseline model covariates (excluding treatment and visit; baseline risk score) or the baseline model covariates*time interactions (slope risk score). The risk scores will be included as covariates in the model.

To calculate the baseline risk score, the GST change score is regressed on the covariates listed below. Individual coefficients for the baseline risk score are multiplied by individual covariate values and summed. A separate slope risk score is calculated for baseline covariates interacting with time.

The MMRM with primary outcome CFB value as the response variable will include the following covariates and fixed effects:

- baseline risk score:
  - baseline score for GST
  - age
- slope risk score:
  - baseline score for GST * time
  - age*time
- Time (continuous)
- Time by treatment interaction (time*treatment)
- baseline score for parameter of interest (if not in GST)
- site pooled by region (random effect)

Model assumptions will be evaluated. If the parametric assumptions are evaluated as inappropriate or violated, rank analogues will be advanced.

All secondary and exploratory endpoints CFB values will be analyzed using the MMRM described above. This analysis will assess whether or not there is a difference in estimated CFB values between treatment group and placebo at the 11-month model estimate using least squares means estimates from the MMRM model.

Speech measurement reporting will be analyzed by a third party vendor. A separate report will be provided.

Responder analyses will be run on MVO2 (primary), GST, T1MW, Peak Workload, mFARS, and CGI-C (functional assessment). Responder analyses are performed to illustrate the clinical meaningfulness of the relationship between treatment differences and efficacy outcomes on a per

person basis. Responder analyses will be assessed at change from baseline cutoffs at the overall quartiles for each of the three variables. Patients who die or are unable to perform the test during the study will be counted as non-responders. Patients who do not complete will be carried forward using the last observation carried forward and the imputed value at the study endpoint will be used for the responder analysis.

In addition to summarizing the results for each relationship between individual clinical responses and treatment multiple clinical responses will also be correlated with treatment using the five GST component outcomes individually. Those who experienced the following combined clinical responses will be summarized in terms of counts and percent:

- Improvement or stabilization on at least 1, 2, 3, 4, and all of the 5 responder endpoints.

The PK of D2-LA and H2-LA will be characterized in terms of minimum and maximum exposure at steady state, based on a validated assay. The PK of D2-LA and H2-LA in RBC, D2-ARA, and H2-ARA also will be characterized in terms of minimum and maximum exposure at steady state; the validation of the assays to characterize these concentration values has not yet been validated.

Full details of planned analyses will be provided in a separate statistical analysis plan.

## 14.2. Determination of Sample Size

For the primary analysis of peak workload, group sample sizes of 30 and 30 achieve over 99% power to reject the null hypothesis of equal means when the population mean difference is $\mu 1 - \mu 2 = 7.5 - -8.3 = 15.8$ with a standard deviation for both groups of 11.1 and with a significance level (alpha) of 0.05 using a two-sided two-sample equal-variance t-test.

Subjects who discontinue from the study may be replaced.

## 14.3. Interim Analysis

One of two interim analyses may be performed. Retrotope will determine which interim, if either, to perform.

**Interim analysis 1 using Month 4 data.**

An interim analysis may be performed when all subjects have completed the Month 4 visit.

The Interim Analysis Committee (IAC) will report the following information to the steering committee, which will report the information to Retrotope management.

- The average within-treatment-group standard deviation of the change from baseline to Month 4 in CPET
- The average within-treatment-group standard deviation of the change from baseline to Month 4 in mFARS

That is, for CPET and for mFARS, the IAC statistician will calculate the within-treatment-group standard deviation of the change from baseline to Month 4 for each treatment group, and then calculate the average of the standard deviations of the two treatment groups.

The IAC will not report the mean change from baseline within treatment group and will not report the difference between the treatment groups in the mean change from baseline.

Retrotope may use this information on average within-treatment-group standard deviation to consider stopping the trial for futility or to consider sample size re-estimation.

The Interim Analysis Committee (IAC) will report the Pearson and Spearman correlation of the change in CPET with the change in mFARS at 4 months, and at the last available time point for each subject. Retrotope may use this information to re-order the primary and first secondary analyses.

There will be no alpha penalty for this interim analysis for the following reasons.

1. Only the average within-treatment-group standard deviations will be reported.
2. Neither mean change from baseline within treatment group nor the difference between the treatment groups in the mean change from baseline will be reported to Retrotope.
3. Retrotope will remain blinded to treatment assignments.

**Interim analysis 2 using Month 11 data.**

An interim analysis may be performed when approximately half the subjects have completed the Month 11 visit.

The Interim Analysis Committee (IAC) will report the following information to the steering committee, which will report the information to Retrotope management.

- The results of a futility analysis for the CPET endpoint
- The results of a futility analysis for the mFARS endpoint

Retrotope may use this information to consider stopping the trial for futility, to change the order of the primary and secondary endpoint, or to consider sample size re-estimation.

The Interim Analysis Committee (IAC) will report the Pearson and Spearman correlation of the change in CPET with the change in mFARS at 4 months, and at the last available time point for each subject. Retrotope may use this information to re-order the primary and first secondary analyses.

There will be no alpha penalty for this interim analysis for the following reasons.

1. The trial cannot be stopped for efficacy at this interim. It can only be stopped for futility.

Retrotope will remain blinded to treatment assignments.

## 14.4. Deviation from Original Analysis Plan

Deviations from the original statistical or PK analysis plans will be provided in the final clinical study report (CSR).

# 15. ADVERSE EVENTS

All subjects in the study will have FRDA and the Investigators will take the subjects' Baseline status into account when reporting AEs. Investigators will also consider the underlying disease when designating relationship of AE to study drug, and if AEs are due to the FRDA disease, disease progression or symptomatology exacerbation. Common characteristics of FRDA and RT001 treatment include the following:

- loss of coordination (ataxia) in the arms and legs
- loss of mobility
- fatigue - energy deprivation and muscle loss
- vision impairment, hearing loss, and slurred speech
- severe scoliosis
- foot deformities such as clubfoot, flexion of the toes, hammer toes, foot inversion
- diabetes mellitus (insulin-dependent, in most cases)
- hypertrophic cardiomyopathy
- chest pain, shortness of breath, and heart palpitations
- myocardial fibrosis
- heart rhythm abnormalities
- steatorrhea, diarrhea, loose stools

All adverse physical findings/laboratory results identified prior to initiation of study drug will be reported on the medical history and/or physical examination CRFs. After initiation of study drug, any clinically significant changes in physical findings/laboratory results or any new adverse physical findings/laboratory results will be reported as AEs.

## 15.1. Adverse Event Definitions

### 15.1.1. Adverse Event

An AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug and does not imply any judgment about causality.

### 15.1.2. Suspected Adverse Reaction

A suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of Investigational New Drug (IND) safety reporting, ‘‘reasonable possibility’’ means there is evidence to suggest a causal relationship between the

drug and the AE. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

### 15.1.3. Life-Threatening Adverse Event or Life-Threatening Suspected Adverse Reaction

An AE or suspected adverse reaction is considered ''life-threatening'' if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

### 15.1.4. Serious Adverse Event or Serious Suspected Adverse Reaction

An AE or suspected adverse reaction is considered ''serious'' if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening AE - see definition above
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

### 15.1.5. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An AE or suspected adverse reaction is considered ''unexpected'' if it is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the Investigator Brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the Investigator Brochure listed only cerebral vascular accidents.

''Unexpected,'' as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the Investigator Brochure as occurring with a class of drugs or as anticipated

from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

## 15.2. Adverse Event Classification

### 15.2.1. Relationship to Investigational Drug

The Investigator's assessment of causality must be provided for all AEs (serious and non-serious). An Investigator's causality assessment is the determination of whether or not there exists a reasonable possibility that the study drug caused or contributed to an AE, as described below:

- **None:** No relationship between the experience and the administration of study drug; related to other etiologies such as concomitant medications or subject's clinical state.
- **Unlikely:** The current state of knowledge indicates that a relationship is unlikely.
- **Possible:** A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the subject's clinical state or other modes of therapy administered to the subject, but this is not known for sure.
- **Probable:** A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.
- **Definite:** A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug and can be confirmed with a positive re-challenge test or supporting laboratory data.

As this is a blinded study, it is assumed the event is related to the active study drug until proven otherwise.

### 15.2.2. Severity

All AEs will be graded for severity using the following terms:

| | | |
|---|---|---|
| **Grade 1** | Mild | Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated |
| **Grade 2** | Moderate | Minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL[a] |
| **Grade 3** | Severe | Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b] |
| **Grade 4** | Life threatening consequences | Urgent intervention indicated |
| **Grade 5** | Death | Death related to an AE |

[a] Instrumental activities of daily life (ADL) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[b] Self-care activities of daily life refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

## 15.3. Exposure *in Utero*

The subject will be instructed to notify the Investigator if the subject or subject's partner becomes pregnant during the study. The Investigator must notify the Sponsor within 24 hours via telephone or e-mail and must complete the Pregnancy Notification Form and submit it to the Sponsor within 2 working days of being notified. The Investigator should obtain informed consent/assent from the subject or subject's partner allowing the Investigator to obtain information regarding the pregnancy and its outcome. If the subject or subject's partner provides informed consent/assent, the Investigator should follow the pregnancy until outcome. A final Pregnancy Notification Form should be completed when the outcome of the pregnancy is known.

## 15.4. Monitoring of Adverse Event Data

The Investigator and the Sponsor or its representative will be responsible for the following:

- Reviewing AE data on an ongoing basis throughout the study. Should any AE report suggest that an unexpected Grade 3 or higher, and probably or definite study drug-related occur, the Investigator and Sponsor will take prompt and appropriate action to protect subject safety. These actions may range from no action (proceed with the study) to halting the study for safety reasons. The evaluation and action decided upon will be documented.
- Assessing the safety data and providing recommendations if the Sponsor should stop or modify the study.

See Section 8.4 for additional details.

## 15.5. Documentation of Adverse Events by Investigator

Subjects will be evaluated and questioned generally to identify AEs during the course of the study. Any events occurring prior to administration of the first dose will be recorded on the Medical History CRF. Events occurring after administration of the first dose of study drug will be recorded on the Adverse Event CRF. Adverse events that occur up to and including the 90 days after the last dose of study drug must be reported.

Record all AEs spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures on the Adverse Event Form for that visit. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE and must be recorded on the Adverse Event Form. In addition, an abnormal test finding must be classified as an AE if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy. (Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE.)
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study.
- The test finding is considered an AE by the Investigator.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, it should be recorded as a separate AE. Laboratory data are to be collected as stipulated in this protocol. Clinical syndromes associated with laboratory abnormalities are to be recorded as appropriate (e.g., diabetes mellitus rather than hyperglycemia).

For SAEs, a Serious Adverse Event Form must also be completed with as much information as possible and submitted in the time frame described below in Section 15.6. When new significant information is obtained as well as when the outcome of an event become known, the Investigator should record the information on a new Serious Adverse Event Form, indicating that it is a follow-up report. If the subject was hospitalized, a copy of the discharge summary and any other relevant hospital records (e.g., admission report, laboratory test results) must be included as part of the subject medical file.

All AEs considered to be related (definitely, probably, or possibly related) to study drug and all SAEs will be followed until resolved or until a stable status has been achieved.

## 15.6. Notification about Serious Adverse Events and Serious and Unexpected Suspected Adverse Reactions

### 15.6.1. Investigator Reporting to Sponsor

All SAEs that occur during the course of the study must be reported by the Investigator to the Sponsor and to the Medical Monitor within 24 hours by telephone or by text message. Additionally, the SAE Form should be faxed/e-mailed within 1 working day from the point in time when the Investigator becomes aware of the SAE. In addition, all SAEs that occur up to and including 90 days after administration of the last dose of study drug must be reported to the Sponsor within 1 working day from when the Investigator becomes aware of the SAE.

Investigators must report to the Sponsor any SAE, whether or not considered drug related, including those listed in the protocol or Investigator Brochure. The report must include an assessment of causality.

For all SAEs, the Investigator is obligated to obtain and provide information to the Sponsor in accordance with the timeframes for reporting specified above. In addition, an Investigator may be requested by the Sponsor to obtain specific additional follow-up information in an expedited fashion. This information may be more detailed than that captured on the Adverse Event eCRF. In general, this will include a description of the AE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to the Sponsor or its designated representative.

The Sponsor's Medical Monitor will review the information submitted and seek further detail if deemed medically relevant and ideally concur on the severity and relatedness. However, the Medical Monitor may revise the severity and relatedness **upward** but may not reduce severity and/or relatedness. Such revisions will be documented in the Sponsor file and provided to the Investigator.

**Serious Adverse Event Reporting Contact Information:**

Mark Midei, MD
Medical Monitor
Retrotope, Inc.
4300 El Camino Real, Suite 201
Los Altos, CA 94022
Tel: 410-371-5357
mark@retrotope.com

### 15.6.2. Reporting to Regulatory Agencies and Institutional Review Boards

If there is a serious and unexpected suspected adverse reaction, the Sponsor will notify the appropriate regulatory agency and all appropriate parties on an expedited basis. In addition, Sponsor must submit expedited reports of an increased rate of occurrence of serious suspected adverse reactions over that listed in the protocol or Investigator Brochure (§ 312.32(c)(1))(iv)).

It is the responsibility of the Investigator to promptly notify the Institutional Review Board (IRB) of all serious and unexpected suspected adverse reactions involving risk to human subjects. Provide a copy of this communication to the Sponsor.

## 15.7. Emergency Identification of Study Medication

In the event of a medical emergency, when knowledge of the subject's treatment assignment may influence the subject's clinical care, the Investigator may obtain the treatment assignment of the subject experiencing the emergency through the unblinded statistician/designee. However, prior to unblinding, the Investigator should make every effort to contact the Medical Monitor.

If unblinding is necessary, the Investigator must document the reasons for unblinding in the subject's source documents but should not divulge the subject's treatment assignment to any individuals except the Medical Monitor (if required) and those individuals involved in the direct care of the subject. The date and the reasons for breaking the blind must be submitted to the Sponsor within 24 hours.

If a subject's treatment assignment is unblinded, the subject should remain in the study and continue the protocol-specified follow-up evaluations.

## 15.8. Optional Sub-Study Adverse Event Monitoring

For the Optional PK Profile Sub-Study, there will be adverse event monitoring because this study will start and stop within the RT001-006 study dosing window.

For the Optional PK Washout Sub-Study, there will not be any adverse event monitoring because the study occurs after the last dose of study drug. SAEs will still be monitored.

## 15.9. Emergency Sponsor Contact

In a medical emergency (such as an event that requires immediate attention regarding the treatment of a subject, operation of the clinical study, and/or the use of investigational drug), study site staff will apply appropriate medical intervention according to current standards of care, and contact the Medical Monitor and the Sponsor Contact.

| **Medical Monitor and Sponsor Contact Information:** |
|---|
| Mark Midei, MD<br>Medical Monitor<br>Retrotope, Inc.<br>4300 El Camino Real, Suite 201<br>Los Altos, CA 94022<br>Tel: 410-371-5357<br>mark@retrotope.com<br><br>Frederic Heerinckx<br>VP, Clinical Operations<br>Retrotope, Inc.<br>4300 El Camino Real, Suite 201<br>Los Altos, CA 94022<br>Tel: 408-834-5729<br>frederic@retrotope.com |

# 16. ETHICS

## 16.1. Institutional Review Board

The IRB must comply with FDA requirements governing IRBs (21 CFR Part 56).

The Investigator will provide the Sponsor (or designee) with documentation of IRB approval of the following documents before the study begins at the study site(s): protocol, ICF, and any other relevant materials intended for or directed to subjects (e.g., subject diaries, advertisements). The Investigator will supply documentation to the Sponsor of IRB requirements regarding continuing review and approval of revisions to any of these documents.

## 16.2. Ethical Conduct of the Study

This study will be conducted in accordance with the current IRB approved clinical protocol, International Conference for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines, and relevant policies and requirements of the national regulations and laws, including the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

## 16.3. Subject Information and Informed Consent

Written informed consent/assent is required from each subject prior to any testing under this protocol, including screening tests and evaluations. The ICF, as specified by the clinical site's IRB/IEC, must follow the Protection of Human Subjects regulations listed in 21 CFR Part 50.

The ICF will be used to explain the risks and benefits of study participation in simple terms before the subject will be entered into the study. The ICF will contain a statement that the consent is freely given, that the subject is aware of the risks and benefits of entering the study, and that the subject is free to withdraw from the study at any time. Written informed consent must be given by the subject after the receipt of detailed information on the study and sufficient time to read it and have any questions answered. It is the responsibility of the Investigator to obtain consent/assent and to provide the subject with a copy of the signed and dated ICF. Confirmation of a subject's informed consent must also be documented in the subject's medical record prior to any testing under this protocol, including screening tests and evaluations.

All ICFs used in this study must be approved by the appropriate IRB and by the Sponsor or its designee. The ICF must not be altered without the prior agreement of the relevant IRB and the Sponsor.

# 17. STUDY ADMINISTRATION

## 17.1. Administrative Structure

A list of individuals who will have key positions in this study will be saved in the Trial Master File (TMF). This list will include names, titles, and roles of selected individuals from the Sponsor and/or the contract research organization (CRO) that will contribute to this study.

## 17.2. Quality Control and Quality Assurance

### 17.2.1. Overview

According to the GCP Guidelines, the Sponsor is responsible for implementing and maintaining quality assurance and control systems with written standard operating procedures (SOPs).

Quality Control (QC) will be applied to each stage of data handling. The following steps will be taken to ensure the accuracy, consistency, completeness, and reliability of the data:

- Investigator meeting(s)
- Site initiation visit
- Routine site monitoring
- Ongoing site communication and training
- Data management quality control checks
- Continuous data acquisition and cleaning
- Internal review of data; and
- QC checks of the final CSR

In addition, the Sponsor's (or designee) Clinical Quality Assurance (QA) Department may conduct periodic audits of the study processes, including, but not limited to study site, site visits, vendors, clinical database, and final CSR. When audits are conducted, access must be authorized to Sponsor's representatives and regulatory authorities for all study-related documents, including medical history and concomitant medication documentation.

### 17.2.2. Monitoring

Site Monitors will work in accordance with Sponsor SOPs. Monitors will establish and maintain regular contact between the Investigator or designee and the Sponsor.

Monitors will evaluate the competence of each study site, informing the Sponsor about any problems relating to facilities, technical equipment or medical staff. During the study, monitors will check that written informed consent/assent has been obtained from all subjects correctly and that data are recorded correctly and completely on the CRFs. Monitors are also required to

compare entries in CRFs with corresponding source data and to inform the Investigator or designee of any errors or omissions. Monitors will also review adherence to the protocol and to regulatory requirements at the study site and discuss any deviations noted with the Investigator or designee. They will arrange for the study site to receive adequate supply of study drug and ensure appropriate storage conditions are maintained.

Monitoring visits will be conducted according to the US CFR Title 21 Parts 50, 56, and 312 and ICH Guideline for GCP. The monitor will make written reports to the Sponsor following each contact with the Investigator or designee, regardless of whether it is by phone or in person.

### 17.2.3. Data Management/Coding

Study data will be handled according to the relevant SOPs of the data management and biostatistics departments of the Sponsor or CRO.

Adverse events will be coded using MedDRA and medications will be coded using World Health Organization Drug Dictionary (WHODD).

### 17.2.4. Quality Assurance Audit

Study sites, the study database, and study documentation may be subject to a Quality Assurance audit by the Sponsor or designee on behalf of the Sponsor. In addition, inspections may be conducted by regulatory bodies at their discretion.

## 17.3. Data Handling and Recordkeeping

### 17.3.1. Electronic Data

When using electronic trial data handling and/or remote electronic trial data systems, the Sponsor will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance (i.e., validation).
- Maintain SOPs for using these systems.
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail).
- Maintain a security system that prevents unauthorized access to the data.
- Maintain a list of the individuals who are authorized to make data changes.
- Maintain adequate backup of the data.

- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing).

Documentation regarding electronic systems used in this protocol is available upon request from the CRO maintaining the electronic trial data system.

### 17.3.2. Case Report Form Completion

CRFs will be completed for each study subject. It is the Investigator's responsibility to ensure the accuracy, completeness, legibility, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status.

Electronic data capture will be used for the study. Data will be recorded on source documentation at each study location and entered electronically by the study center personnel. Data collected on each subject will be documented on the appropriate eCRF. Completed eCRFs are to be signed off by the Investigator or his/her designee.

### 17.3.3. Data Handling

If data are transformed during processing, records will be maintained so that it will be possible to compare the original data and observations with the processed data.

An unambiguous subject identification code will be used that allows identification of all the data reported for each subject.

### 17.3.4. Retention of Study Records

The Investigator must maintain essential study documents (protocol and protocol amendments, completed CRFs, signed ICFs, relevant correspondence, and all other supporting documentation) until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years after the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or the hospital, institution, or private practice in which the study is being conducted. Subject identification codes (subject names and corresponding study numbers) will be retained for this same period of time. These documents may be transferred to another responsible party, acceptable to the Sponsor, who agrees to abide by the retention policies. Written notification of transfer must be submitted to the Sponsor. The Investigator or designee must contact the Sponsor prior to disposing of any study records.

## 17.4. Financing and Insurance

Financing and insurance are addressed in a separate document.

## 17.5. Confidentiality

To maintain subject privacy, all CRFs, study drug accountability records, study reports and communications will identify the subject by the assigned subject number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Subject will be notified that registration information, results, and other information about this study will be submitted to ClinicalTrials.gov, a publicly available trial registry database; however, protected health information of individual subjects will not be used.

All information regarding the investigational product supplied by the Sponsor to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used toward the development of the investigational product and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

## 17.6. Publication Policy

The data generated by this study are considered confidential information and the property of Sponsor and shall not be published or disclosed without the prior written consent of Sponsor.

## 17.7. Direct Access to Source Data

The Investigators/institutions/clinical sites will permit trial-related monitoring, audits, IRB review, and regulatory inspections as requested by FDA, the Sponsor, or the Sponsor designee, including direct access to source data/documents (e.g., original medical records, laboratory reports, hospital documents, progress reports, signed informed consent forms) in addition to CRFs.

The Investigator or designee will prepare and maintain adequate and accurate source documents to support all observations and other pertinent data recorded on the CRFs for each subject randomized into the study.

The Investigator will allow the Sponsor (or designee), and authorized regulatory authorities to have direct access to all documents pertaining to the study.

## 17.8. Protocol Amendments

Changes to the conduct of the study should be prepared as a protocol amendment and implemented only upon approval of the Sponsor, or a representative of the Sponsor. Protocol amendments should also receive written IRB approval prior to implementation, except when necessary to eliminate immediate hazards to the subjects or when the changes involve only logistical or administrative aspects of the trial (e.g., change of monitor, telephone numbers). In this case, the Sponsor will amend and implement the protocol change and subsequently notify the regulatory authorities and/or the IRB, as appropriate.

# 18. REFERENCES

Abeti R, Parkinson MH, Angelova PR, Hargreaves IP, Sandi C, Pook MA, et al. Mitochondrial energy imbalance induces oxidative stress and cell death in a Friedreich's ataxia. Pharmacol. Res. 2015, 99:344-350

Abozguia K, Elliott P, McKenna W, et al. Metabolic modulator perhexiline corrects energy deficiency and improves exercise capacity in symptomatic hypertrophic cardiomyopathy. Circulation 2010; 122:1562-9.

Abraham WT, Fisher WG, Smith AL, Delurgio DB, Leon AR, Loh E, Kocovic DZ, Packer M, Clavell AL, Hayes DL, Ellestad M. New England Journal of Medicine. 2002 Jun 13;346(24):1845-53.

Agostoni P, Bussotti M, Cattadori G, Margutti E, Contini M, Muratori M, Marenzi G, Fiorentini C. Gas diffusion and alveolar-capillary unit in chronic heart failure. Eur Heart J. 2006; 27:2538 – 2543

Andreyev AY, Tsui HS, Milne GL, Shmanai VV, Bekish AV, Fomich MA, et al. Isotope-reinforced polyunsaturated fatty acids protect mitochondria from oxidative stress. Free Radic Biol Med. 2015 Jan 8. pii: S0891-5849(15)00003-9.

Arena R, Owens DS, Arevalo J, et al. Ventilatory efficiency and resting hemodynamics in hypertrophic cardiomyopathy. Medicine and science in sports and exercise. 2008; 40:799-805.

Arena R, Lavie CJ, Milani RV, Myers J, Guazzi M. Cardiopulmonary exercise testing in patients with pulmonary arterial hypertension: an evidence-based review. The Journal of Heart and Lung Transplantation. 2010 Feb 28;29(2):159-73.

Armstrong S, Khdour O, Hecht SM. Does oxidative stress contribute to the pathology of Friedreich's ataxia? A radical question. FASEB J. 2010 Jul;24(7):2152-63. Epub 2010 Mar 10. PubMed PMID: 20219987.

Artuch R, Aracil A, Mas A, Colome C, Rissech M, Monros E, Pineda M. Friedreich's ataxia: idebenone treatment in early stage patients. Neuropediatrics. 2002 Aug;33(04):190-3.

Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, Forman D, Franklin B, Guazzi M, Gulati M, Keteyian SJ. Clinician's guide to cardiopulmonary exercise testing in adults. Circulation. 2010 Jul 13;122(2):191-225.

Brenna JT. Use of stable isotopes to study fatty acid and lipoprotein metabolism in man. Prostaglandins Leukot Essent Fatty Acids. 1997 Oct;57(4-5):467-72. PubMed PMID: 9430398.

Brubaker PH, Marburger CT, Morgan TM, et al. Exercise responses of elderly patients with diastolic versus systolic heart failure. Medicine and science in sports and exercise. 2003; 35:1477-85.

Burr GO, Burr MM. A new deficiency disease produced by the rigid exclusion of fat from the diet. J Biol Chem. 1929 (82):345-367.

Burr GO, Burr MM. On the nature and role of the fatty acids essential in nutrition. J Biol Chem. 1930 (86):587-621.

Chen CT, Green JT, Orr SK, Bazinet RP. Regulation of brain polyunsaturated fatty acid uptake and turnover. Prostaglandins Leukot Essent Fatty Acids 2008; 79: 85–91.

Chen LF, Wang CH, Chou PC, Ho SC, Joa WC, Sheng TF, Kuo HP. Association between emphysema score, six-minute walk and cardiopulmonary exercise tests in COPD. Open Respir Med J. 2012 Jan 1; 6:104-10

Coats CJ, Rantell K, Bartnik A, Patel A, Mist B, McKenna WJ, Elliott PM. Cardiopulmonary exercise testing and prognosis in hypertrophic cardiomyopathy. Circ Heart Fail. 2015; 8:1022-1031

Cohn JN, Johnson GR, Shabetai R, Loeb H, Tristani F, Rector T, Smith R, Fletcher R. Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias, and plasma norepinephrine as determinants of prognosis in heart failure: the V-HeFT VA Cooperative Studies Group.Circulation 1993; 87 (suppl): V-I5–V-16

Davies LC, Francis DP, Piepoli M, Scott AC, Ponikowski P, Coats AJ. Chronic heart failure in the elderly: value of cardiopulmonary exercise testing in risk stratification. Heart 2000; 83:147–151

Davies LC, Wensel R, Georgiadou P, Cicoira M, Coats AJ, Piepoli MF, Francis DP. Enhanced prognostic value from cardiopulmonary exercise testing in chronic heart failure by non-linear analysis: oxygen uptake efficiency slope. European heart journal. 2006; 27:684-90.

DeBrosse C, Nanga RP, Wilson N, et al. Muscle oxidative phosphorylation quantitation using creatine chemical exchange saturation transfer (CrCEST) MRI in mitochondrial disorders. JCI insight. 2016;1(18)

Diller GP, Giardini A, Dimopoulos K, Gargiulo G, Müller J, Derrick G, Giannakoulas G, Khambadkone S, Lammers AE, Picchio FM, Gatzoulis MA. Predictors of morbidity and mortality in contemporary Fontan patients: results from a multicenter study including cardiopulmonary exercise testing in 321 patients. European heart journal. 2010 Oct 7: ehq356

Durr A, Cossee M, Agid Y, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. N Engl J Med 1996; 335:1169 –75

Duscha BD, Kraus WE, Keteyian SJ, Sullivan MJ, Green HJ, Schachat FH, Pippen AM, Brawner CA, Blank JM, Annex BH. Capillary density of skeletal muscle: a contributing mechanism for exercise intolerance in class II–III chronic heart failure independent of other peripheral alter- ations. J Am Coll Cardiol. 1999;33:1956 –1963

Duscha BD, Schulze PC, Robbins JL, Forman DE. Implications of chronic heart failure on peripheral vasculature and skeletal muscle before and after exercise training. Heart Fail Rev. 2008; 13:21–37

Emken EA, Adlof RO, Rakoff H, Rohwedder WK, Gulley RM. Metabolism in vivo of deuterium-labelled linolenic and linoleic acids in humans. Biochem Soc Trans. 1990 Oct;18(5):766-9. PubMed PMID: 2083671.

Farr MJ, Lang CC, LaManca JJ, et al. Cardiopulmonary exercise variables in diastolic versus systolic heart failure. The American journal of cardiology. 2008; 102:203-6.

Flaherty KR, W J, Weisman IM, Zeballos RJ, Schork MA, Blaivas M, Rubenfire M, Martinez FJ. Unexplained exertional limitation: characterization of patients with a mitochondrial myopathy. American journal of respiratory and critical care medicine. 2001 Aug 1;164(3):425-32.

Foury F, Cazzalini O. Deletion of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria. FEBS Lett. 1997 Jul 14;411(2-3):373-7. PubMed PMID: 9271239.

Francis DP, Shamim W, Davies LC, Piepoli MF, Ponikowski P, Anker SD, Coats AJ. Cardiopulmonary exercise testing for prognosis in chronic heart failure: continuous and independent prognostic value from VE/VCO 2 slope and peak VO 2. European Heart Journal. 2000 Jan 1;21(2):154-61.

Grazia Cotticelli M, Crabbe AM, Wilson RB, Shchepinov MS. Insights into the role of oxidative stress in the pathology of Friedreich ataxia using peroxidation resistant polyunsaturated fatty acids. Redox Biology. 2013 (1):398-404.

Guazzi M, Myers J, Peberdy MA, Bensimhon D, Chase P, Arena R. Exercise oscillatory breathing in diastolic heart failure: prevalence and prognostic insights. European Heart Journal. 2008;4; 29:2751-9.

Guéraud F, Atalay M, Bresgen N, Cipak A, Eckl PM, Huc L, et al. Chemistry and biochemistry of lipid peroxidation products. Free Radic Res. 2010 Oct;44(10):1098-124. PubMed PMID: 20836659.

Halliwell B. Oxidative stress and neurodegeneration: where are we now? J Neurochem 2006; 97: 1634–1658.

Hambrecht R, Fiehn E, Yu J, et al. Effects of endurance training on mitochondrial ultra- structure and fiber type distribution in skeletal muscle of patients with stable chronic heart failure. J Am Coll Cardiol. 1997; 29:1067–73

Harding AE. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain 1981; 104:589–620

Heden TD, Morris EM, Kearney ML, Liu TW, Park YM, et al. Differential effects of low-fat and high-fat diets on fed-state hepatic triacylglycerol secretion, hepatic fatty acid profiles, and DGAT-1 protein expression in obese-prone Sprague-Dawley rats. Appl Physiol Nutr Metab. 2014 Apr;39(4):472-9. PubMed PMID: 24669989.

Hewer RL. Study of fatal cases of Friedreich's ataxia. Brit Med J 1968; 3:649 – 52

Hill S, Hirano K, Shmanai VV, Marbois BN, Vidovic D, Bekish AV, et al. Isotope-reinforced polyunsaturated fatty acids protect yeast cells from oxidative stress. Free Radic Biol Med. 2011 Jan 1;50(1):130-8. PubMed PMID: 20955788.

Hill S, Lamberson CR, Xu L, To R, Tsui HS, Shmanai VV, et al. Small amounts of isotope-reinforced polyunsaturated fatty acids suppress lipid autoxidation. Free Radic Biol Med. 2012 Aug 15;53(4):893-906. PubMed PMID: 22705367.

Hunt SA, Baker DW, Chin MH, et al. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary: A report of the American college of Cardiology/American Heart Association Task Force on Practice Guidelines. J Amer Coll Cardiol 2001; 38:2101-13.

International Atomic Energy Agency (IAEA). IAEA Human Health Series No. 3. Assessment of Body Composition and Total Energy Expenditure in Humans Using Stable Isotope Techniques. 2009.

Intralipid®. Prescribing Information. 2006. Fresenius Kabi. Rev June 2006.

Jeppesen TD, Schwartz M, Olsen DB, Vissing J. Oxidative capacity correlates with muscle mutation load in mitochondrial myopathy. Ann Neurol. 2003; 54:86 –92

Kleber FX, Vietzke G, Wernecke KD, Bauer U, Opitz C, Wensel R, Sperfeld A, Glaser S. Impairment of ventilatory efficiency in heart failure: prognostic impact. Circulation 2000; 101:2803–2809

Lamberson CR, Xu L, Muchalski H, Montenegro-Burke JR, Shmanai VV, Bekish AV, et al. Unusual kinetic isotope effects of deuterium reinforced polyunsaturated fatty acids in tocopherol-mediated free radical chain oxidations. J Am Chem Soc. 2014 Jan 22;136(3):838-41. PubMed PMID: 24380377.

Lewis GD, Shah R, Shahzad K, Camuso JM, Pappagianopoulos PP, Hung J, Tawakol A, Gerszten RE, Systrom DM, Bloch KD, Semigran MJ. Sildenafil improves exercise capacity and quality of life in patients with systolic heart failure and secondary pulmonary hypertension. Circulation. 2007 Oct 2;116(14):1555-62.

Lodi R, Rajagopalan B, Blamire AM, et al. Cardiac energetics are abnormal in Friedreich ataxia patients in the absence of cardiac dysfunction and hypertrophy: an in vivo 31P magnetic resonance spectroscopy study. Cardiovasc Res 2001; 52:111-9

Lynch DR, Farmer JM, Tsou AY, Perlman S, Subramony SH et al. Measuring Friedreich ataxia: complementary features of examination and performance measures. Neurology 2006; 66:1711-1716.

Malinska D, Kudin AP, Debska-Vielhaber G, Vielhaber S, Kunz WS. Chapter 23 Quantification of superoxide production by mouse brain and skeletal muscle mitochondria. Methods Enzymol 2009; 456: 419–437.

Marin-García J, Goldenthal MJ, Moe GW. Abnormal cardiac and skeletal muscle mitochondrial function in pacing-induced cardiac failure. Cardiovasc Res. 2001; 52:103–110

Martelli A, Napierala M, Puccio H. Understanding the genetic and molecular pathogenesis of Friedreich's ataxia through animal and cellular models. Dis Model Mech. 2012 Mar;5(2):165-76. PubMed PMID: 22382366.

Mettauer B, Zoll J, Garnier A, Ventura-Clapier R. Heart failure: a model of cardiac and skeletal muscle energetic failure. Pflugers Arch. 2006; 452:653– 666

Miyamoto S, Nagaya N, Satoh T, Kyotani S, Sakamaki F, Fujita M, Nakanishi N, Miyatake K. Clinical correlates and prognostic significance of six-minute walk test in patients with primary

pulmonary hypertension: comparison with cardiopulmonary exercise testing. American journal of respiratory and critical care medicine. 2000 Feb 1;161(2):487-92

Mottram PM, Delatycki MB, Donelan L, et al. Early changes in left ventricular long-axis function in Friedreich ataxia: relation with the FXN gene mutation and cardiac structural change. J Am Soc Echocardiogr 2011; 24:782–9

Mudge GH, Goldstein S, Addonizo LJ, Caplan A, Mancini D, Levine TB, Ritsch ME Jr, Stevenson LW. 24th Bethesda Conference Taskforce 3: Heart transplantation: Recipient guidelines/prioritization. J Am Coll Cardiol 1993; 22:21–31

National Academy of Sciences: Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council. Nutrient Requirements of Laboratory Animals. Fourth Revised Edition. National Academy Press. Washington, DC. 1995.

Papazachou O, Anastasiou-Nana M, Sakellariou D, Tassiou A, Dimo- poulos S, Venetsanakos J, Maroulidis G, Drakos S, Roussos C, Nanas S. Pulmonary function at peak exercise in patients with chronic heart failure. Int J Cardiol. 2007; 118:28 –35

Patel M, Isaacs CJ, Seyer L. Progression of Friedreich ataxia: quantitative characterization over 5 years. Ann Clin Translat Neurol 2016; 3:684-94

Phan TT, Abozguia K, Shivu GN, et al. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. Journal of the American College of Cardiology. 2009;54:402-9.

Qian SY1, Buettner GR. Iron and dioxygen chemistry is an important route to initiation of biological free radical oxidations: an electron paramagnetic resonance spin trapping study. Free Radic Biol Med. 1999 Jun;26(11-12):1447-56. PubMed PMID: 10401608.

Ross RM, Murthy JN, Wollak ID, Jackson AS. The six-minute walk test accurately estimates mean peak oxygen uptake. BMC pulmonary medicine. 2010 May 26;10(1):31

Salem N Jr, Wegher B, Mena P, Uauy R. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proc Natl Acad Sci U S A. 1996 Jan 9;93(1):49-54. PubMed PMID: 8552667.

Santos R, Lefevre S, Sliwa D, Seguin A, Camadro JM, Lesuisse E. Friedreich ataxia: molecular mechanisms, redox considerations, and therapeutic opportunities. Antioxid Redox Signal. 2010 Sep 1;13(5):651-90. PubMed PMID: 20156111.

Schulz JB, Boesch S, Burk K, et al. Diagnosis and treatment of Friedreich ataxia: a European perspective. Nat Rev Neurol 2009; 5:222–34

Showkathali R. Chapter 9: Use of Cardiopulmonary Exercise Testing in Patients with Heart Failure. In Treadmill Exercise and its Effects. Azoia N, et al 2009; Nova Science:1-7

Siciliano G, Volpi L, Piazza S, Ricci G, Mancuso M, Murri L. Functional diagnostics in mitochondrial diseases. Biosci Rep. 2007;27: 53– 67

Singh SJ, Puhan MA, Andrianopoulos V, et al. An official systematic review of the European Respiratory Society/American Thoracic Society: measurement properties of field walking tests in chronic respiratory disease. Eur Resp J 2014; 44:1447-78

Sival DA, Pouwels ME, Van Brederode A, et al. In children with Friedreich ataxia, muscle and ataxia parameters are associated. Developmental Medicine & Child Neurology. 2011 Jun 1;53(6):529-34.

Sorajja P, Allison T, Hayes C, Nishimura RA, Lam CS, Ommen SR. Prognostic utility of metabolic exercise testing in minimally symptomatic patients with obstructive hypertrophic cardiomyopathy. The American journal of cardiology. 2012; 109:1494-8.

Subramony SH, May W, Lynch D, Gomez C, Fischbeck K, Hallett M, et al. Measuring Friedreich ataxia: Interrater reliability of a neurologic rating scale. Neurology. 2005 Apr 12;64(7):1261-2.

Sullivan MJ, Knight JD, Higginbotham MB, Cobb FR. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure: muscle blood flow is reduced with maintenance of arterial perfusion pressure. Circulation. 1989; 80:769 –781

Taivassalo T, Shoubridge EA, Chen J, et al. Aerobic conditioning in patients with mitochondrial myopathies: physiological, biochemical, and genetic effects. Ann Neurol 2001; 50:133-41.

Taivassalo T, Jensen TD, Kennaway N, DiMauro S, Vissing J, Haller RG. The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients. Brain. 2003; 126:413–423.

Taivassalo T, Haller RG. Exercise and training in mitochondrial myopathies. Med Sci Sports Exerc. 2005; 37:2094 –2101

Tanabe Y, Nakagawa I, Ito E, Suzuki K. Hemodynamic basis of the reduced oxygen uptake relative to work rate during incremental exercise in patients with chronic heart failure. Int J Cardiol. 2002; 83:57– 62

Tsou AY, Paulsen EK, Lagedrost SJ, et al. Mortality in Friedreich ataxia. J Neurol Sci 2011; 307:46–9

Vorgerd M, Schöls L, Hardt C, Ristow M, Epplen JT, Zange J. Mitochondrial impairment of human muscle in Friedreich ataxia in vivo. Neuromuscular Disorders. 2000 Aug 1;10(6):430-5

Weidemann F, Rummey C, Bijnens B, et al. The Heart in Friedreich Ataxia. Definition of cardiomyopathy, disease severity, and correlation with neurological symptoms. Circulation 2012; 125:1626-34

Weidemann F, Störk S, Liu D, Hu K, Herrmann S, Ertl G, Niemann M. Cardiomyopathy of Friedreich ataxia. Journal of neurochemistry. 2013 Aug 1;126(s1):88-93.

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR. Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 2004; 5: 863–873.

Zesiewicz, T. EPI-743 (Alpha-tocotrieneol Quinone) demonstrates long-term improvement in neurological function and disease progression in Friedreich's ataxia, Neurology 2017 v88, no.16, supplement S17.005

# 19. APPENDICES

## 19.1. Cardiopulmonary Exercise Testing as a Primary Endpoint in Mitochondrial Diseases

### 19.1.1. Background

Assessment of exercise capacity is useful in providing valuable diagnostic, prognostic, and therapeutic information in patients in a variety of disease states. Exercise capacity traditionally has been assessed in heart failure by the New York Heart Association criteria. Such assessment is both subjective and insensitive. The 6-minute walk test (i.e., the distance walked over a period of six minutes) is less subjective than the New York Heart Association functional class but still can be heavily influenced by the patient's and/or tester's motivation. Additionally, the results of the 6-minute walk test cannot estimate how close the patient is to his or her maximal capacity.

Some form of quantitative exercise testing has been available for more than a half century and, like many other cardiovascular procedures, has evolved in its technology and scope. Information regarding a patient's ECG and vital sign responses to supervised exercise on a treadmill or bicycle has long been used for diagnostic purposes in clinical situations. It has also been used to predict prognosis, and on this basis, in the qualification of patients for therapeutic interventions. Used after such an intervention, it may be used to assess the resultant effects on symptom status or disease progression. The quantitative assessment a patient's exercise capacity provides a wide array of unique and clinically useful information. Over time, the type of data collected, and the analysis performed during exercise testing has become increasingly complex. This complexity allows the tailoring of the type of exercise protocol used, the method and amount of data collection, and its analysis to the condition under study.

### 19.1.2. What is cardiopulmonary exercise testing (CPET)?

CPET refers to the addition of gas exchange parameter measurement and analysis to conventional supervised exercise testing. This usually entails a protocol administered stress test using either a treadmill or cycle ergometer (for either upper body or lower body exercise). The protocol selected for administration may be tailored for the particular individual or condition under examination. Under such conditions, the peak workload can be measured objectively and reproducibly. Gas exchange analysis at rest, during exercise, and during recovery yields breath-by-breath measures of oxygen uptake ($VO_2$), carbon dioxide output ($VCO_2$), and ventilation (VE). Such metrics add utility to the peak workload analysis and may provide objective evidence of consistent effort when the anaerobic threshold is attained. These data can be readily integrated with standard variables measured during exercise testing, including heart rate, blood pressure, work rate, electrocardiography findings, and symptoms to provide a comprehensive assessment of exercise tolerance and exercise responses.

The ability to perform physical exercise is critically related to the cardiovascular system's capacity to supply oxygen (O2) to the muscles and the pulmonary system's ability to clear carbon dioxide (CO2) from the blood via the lungs. The cardiovascular and respiratory systems work together to provide both a delivery system (of O2) and a removal system (of CO2) from the tissues. There are 4 processes that occur to make this happen:

1. Pulmonary ventilation, or the movement of air into and out of the lungs;
2. Pulmonary diffusion, or the exchange of O2 and CO2 between the lungs and the blood;
3. Transport of O2 and CO2 in the blood;
4. Capillary gas exchange, or the exchange of O2 and CO2 between the capillary blood and the working muscle.

The first 2 processes are referred to as *external respiration* because they involve the movement of gases from the ambient air into the lungs and then the blood. The fourth step is commonly termed *internal respiration* because it involves gas exchange between the blood and the tissues. These 2 processes are linked by the circulatory system. CPET is valuable because taxing the mechanisms responsible for external and internal respiration by exercise can frequently reveal abnormalities not apparent at rest. In addition, typical pulmonary and cardiac function tests performed at rest cannot reliably determine exercise capacity or the particular mechanisms underlying exercise intolerance among individual patients with diseases affecting the heart, the lungs or the muscles (Balady et al. 2010).

Maximal aerobic capacity ($VO_2MAX$) is frequently cited as the single most important derivative of the CPET test because it is considered to be the metric that defines the limits of the cardiopulmonary system. Although $VO_2MAX$ is measured in liters of oxygen per minute, it is usually expressed in milliliters of oxygen per kilogram of body weight per minute to facilitate inter-subject comparisons. The measurement of $VO_2MAX$ implies that an individual's physiological limit has been reached (also termed *maximal aerobic capacity)*. Consistent attainment of this limit is dependent on the efforts of the subject and the administrator of any test of exercise capacity. CPET has an advantage over most of these tests in that maximal effort may be assured reproducibly when the oxygen consumption curve flattens at the anaerobic threshold. This also objectively assures the validity of other performance parameters made during CPET such as measurement of total workload.

### 19.1.3. Conditions in which it is used clinically

CPET may be useful in clinical conditions where exercise limitation occurs as a result of disease. For any appropriate disease in which exercise intolerance is a prominent feature, CPET may help to diagnose, identifying degree or stage, detect progression, predict prognosis, and guide therapeutic intervention.

The value of CPET and its attendant data elements including peak workload and $VO_2MAX$ has been recognized as the most objective method to assess exercise capacity in heart failure patients (Balady et al. 2010). It is a useful test to determine the severity of the disease and to help to determine whether heart failure is the cause of exercise limitation (American College of Cardiology/American Heart Association recommendation Class IIa, Level of Evidence C), provide important prognostic information and identify candidates for cardiac transplantation or other advanced treatments (American College of Cardiology/American Heart Association Recommendation Class IIa, Level of Evidence B); facilitate the exercise prescription (American College of Cardiology/American Heart Association Recommendation Class I, Level of Evidence C) (Hunt et al. 2001); and assess the effectiveness of new drugs and devices. $VO_2MAX$ has been

shown to predict prognosis in patients with heart failure in many studies (Davies et al. 2000, Cohn et al 1993). In addition, there may be enhanced prognostic value from CPET testing in chronic heart failure by non-linear analysis. Use of the oxygen uptake efficiency slope may add to the utility of the CPET testing (Davies et al. 2006).

Peak workload and $VO_2$ MAX during CPET are also useful in the assessment of patients with hypertrophic cardiomyopathy (HCM). In a retrospective review of 182 patients with HCM and minimal or no symptoms followed for up to four years, multiple parameters of metabolic exercise were associated with a risk of death and the development of severe symptoms. On multivariate analyses, the independent predictors of death and severe symptoms were the severity of the left ventricular outflow tract gradient at rest and the percentage of predicted peak myocardial oxygen consumption achieved during exercise. (Sorajja et al. 2012) In a more recent study of 1898 patients with HCM, $VO_2$MAX and several sub-maximal exercise parameters measured during CPET were predictors of death or need for cardiac transplantation (Coats et al. 2015).

CPET parameters are also used clinically in the evaluation of chronic obstructive pulmonary disease. Reductions in $VO_2$MAX, and attainment of anaerobic threshold at lower levels of work performed correlate well with the degree of impairment due to emphysema, and with worse prognosis (Chen et al. 2012).

### 19.1.4. Association with morbidity/mortality

Peak workload measured during exercise testing correlates well with functional capacity. In patients with respiratory disease, peak workload is more closely associated with the distance a patient is able to walk over 6 minutes (6MWD-a common endpoint in heart failure and respiratory failure clinical trials) than it is with tests of respiratory function (Singh et al. 2014). CPET predicts poor outcomes and mortality in a variety of conditions including dilated cardiomyopathies (Mudge et al. 1993, Francis et al. 2000, Davies et al. 2000, Kleber et al. 2000), HCM (Sorraja et al. 2012, Coats et al. 2015), congenital heart disease (Diller et al. 2010), and pulmonary hypertension (Miyamoto et al. 2000).

### 19.1.5. Conditions for which it has been used as an endpoint in clinical trials

CPET is useful in a number of different ways when used as part of a clinical trial. Determinations of peak workload and $VO_2$MAX have been used to qualify subjects objectively for enrollment and assessment of therapeutic effect. Assessment of patient candidacy for enrollment in a clinical trial is fraught with subjectivity among investigators. The use of a $VO_2$MAX threshold may be used to qualify patients for enrollment (Abozguia et al. 2010) and may assure better uniformity, prevents unnecessary drug exposure to patients unlikely to benefit, and would potentially guide later application of perhexiline use clinically.

CPET parameters are continuous variables which may improve trial efficiency by allowing the identification of a significant treatment effect with a smaller number of patients required for efficacy analysis in rare diseases.

In a study of 453 patients with advanced congestive heart failure randomized to control or cardiac resynchronization therapy, $VO_2$MAX was used as a secondary endpoint (Abraham et al.

2002) and correlated well with 6-minute walk distance (primary endpoint). Other studies have shown good correlation between these two endpoints (Ross et al. 2010).

Although the majority of literature supporting the value of CPET in patients with heart failure has been performed in patients with systolic dysfunction, initial investigations demonstrate that CPET also has great promise in the evaluation of patients who have HFNEF—the kind of heart failure responsible for symptoms in HCM patient (Phan et al. 2009). Patients with heart failure resulting from either systolic or diastolic dysfunction appear to have the same degree of impaired aerobic capacity (Brubaker et al. 2003, Farr et al. 2008). In subjects diagnosed with HCM, $VO_2$ MAX and $VCO_2$ MAX at peak exercise are both significantly correlated with resting pulmonary hemodynamics (Arena et al. 2008). An abnormal response effectively identified subjects with pulmonary hypertension. Other investigations indicate the $VCO_2$ MAX slope, and $VO_2$ MAX may be significant predictors of adverse events in heart failure patients with diastolic dysfunction (Arena et al. 2008, Guazzi et al. 2008).

$VO_2$ MAX has been shown in a pilot clinical trial to serve well as a qualifier for enrollment and has been used successfully in determining a significant difference in $VO_2$ MAX (Abozguia et al 2010). Forty-six patients with symptomatic HCM and a $VO_2$ MAX <75% of predicted were enrolled in a trial comparing perhexiline with placebo. At the end of 4.6 $\pm$ 1.8 months, $VO_2$ MAX increased in the perhexiline group (22.2$\pm$0.2 to 24.3$\pm$0.2 mL/kg/min) while it declined in the placebo group (23.6$\pm$0.3 to 22.3$\pm$0.2 mL/kg/min; $p<0.003$). These changes were associated with significant improvement in cardiac energetic status (measured by myocardial ratio of phosphocreatine to adenosine triphosphate using 31P magnetic resonance spectroscopy), improved diastolic function (measured by left ventricular diastolic filling at rest and during exercise using radionuclide ventriculography), and improved quality of life (using NYHA Classification).

Thus, CPET and the parameters extracted from its performance play important roles in identifying patients at highest risk. These metrics serve as continuous variables in the assessment of benefit from pharmacologic intervention in patients with heart failure, and have recently been shown to serve an identical purpose specifically in HCM.

## 19.2. FARS-Neuro Scale

| **A. BULBAR**<br>Most subjects with FA do not have significant facial or tongue atrophy. If mild facial or tongue atrophy is noted score as per instructions. Speech and cough assessment are self-explanatory. For items in Section A, increments of 0.5 may be used if examiner feels an item falls between two severities. | |
|---|---|
| 1. **Facial Atrophy, Fasciculation, Action Myoclonus, and Weakness**<br>0 – None<br>1 – Fasciculations or action myoclonus, but no atrophy.<br>2 – Atrophy present but not profound of complete.<br>3 – Profound atrophy and weakness. | **Score** ☐.☐ |
| 2. **Tongue Atrophy, Fasciculation, Action Myoclonus, and Weakness**<br>0 – None<br>1 – Fasciculations or action myoclonus, but no atrophy.<br>2 – Atrophy present but not profound of complete.<br>3 – Profound atrophy and weakness. | **Score** ☐.☐ |
| **3. Cough** (subject asked to cough forcefully 3 times)<br>0 – Normal.<br>1 – Depressed.<br>2 – Totally or nearly absent. | **Score** ☐.☐ |
| **4. Speech** (ask the patient to read or repeat the sentences: A. "The president lives in the white house" and B. "The traffic is heavy today")<br>0 – Normal.<br>1 – Mild (all or most words understandable).<br>2 – Moderate (most words not understandable).<br>3 – Severe (no or almost no useful speech). | **Score** ☐.☐ |
| **TOTAL BULBAR SCORE:** | ☐☐.☐ |

**B. UPPER LIMB COORDINATION**

Upper limb coordination: Most of the items are self-explanatory. For items 3-5, ask the subject to count as they do the task.

Example: "Move your hand back and forth 10 times as fast as you can. Please count each time to yourself".

You can time the activity with either a watch or a stopwatch. For items in Section B, increments of 0.5 may be used if examiner feels an item falls between two severities.

| | |
|---|---|
| **1. Finger to Finger Test** (Index fingers are placed in front of each other with flexion at the elbow about 25 cm. from the sternum. Observe for 10 seconds. Score amplitude of oscillations)<br>0 – Normal.<br>1 – Mild oscillations of finger (less than 2 cm).<br>2 – Moderate oscillations of finger (2-6 cm).<br>3 – Severe oscillations of finger (greater than 6 cm) | **Score (Right)** ☐.☐<br>**Score (Left)** ☐.☐ |
| **2. Nose-Finger Test** (Assess kinetic or intention tremor during and towards the end of movement: examiner holds index finger at 90% reach of patient; test at least 3 nose-finger-nose trials; movement slow greater than 3 sec.)<br>0 - None<br>1 - Mild (less than 2 cm. amplitude).<br>2 - Moderate (2-6 cm. amplitude or persisting through movement).<br>3 - Severe (greater than 6 cm. & persisting through movement).<br>4 - Too poorly coordinated to perform task. | **Score (Right)** ☐.☐<br>**Score (Left)** ☐.☐ |
| **3. Dysmetria Test** (The subject touches tip of examiner's finger then subject's chin 8 times as rapidly as possible while the examiner moves his finger to four corners of a one-foot square and at about 90% reach of the subject. Assess dysmetria – i.e. inaccuracy of reaching the target- tip of examiner's finger)<br>0 - None.<br>1 - Mild (misses 2 or fewer times).<br>2 - Moderate (misses 3-5 times).<br>3 - Severe (misses 6-8 times.).<br>4 - Too poorly coordinated to perform task. | **Score (Right)** ☐.☐<br>**Score (Left)** ☐.☐ |
| **4. Rapid Alternating Movements of Hands** (Subject should be seated. Forearm pronation/supination 15 cm. above thigh; 10 full cycles as fast as possible; assess rate, rhythm, accuracy; practice 10 cycles before rating, if time greater than 7 sec. add 0.5 to score. Use stopwatch)<br>0 - Normal.<br>1 - Mild (slightly irregular or slowed).<br>2 - Moderate (irregular and slowed).<br>3 – Too poorly coordinated to perform task.<br>**Time to Complete (R):** _____<br>**Time to Complete (L):** _____ | **Score (Right)** ☐.☐<br>**Score (Left)** ☐.☐ |

| | |
|---|---|
| **5. Finger Taps** (index fingertip-to-thumb crease; 15 reps as fast as possible; practice 15 reps once before rating; if time greater than 6 sec., add 1 to rating. Use stopwatch)<br>0 - Normal.<br>1 - Mild (misses 1-3 times).<br>2 - Moderate (misses 4-9 times).<br>3 - Severe (misses 10-15 times).<br>4 - Cannot perform the task.<br>**Time to complete (R):** _____<br>**Time to complete (L):** _____ | **Score (Right)** ☐☐.☐<br>**Score (Left)** ☐☐.☐ |
| **TOTAL UPPER LIMB COORDINATION SCORE** | ☐☐☐.☐ |
| **C. LOWER LIMB COORDINATION**<br>Lower limb coordination: The heel shin slide is scored 1 if there is an abnormality but contact is steady along the top of the shin. If the heel starts going off the shin to one or other side score 2 or 3 as noted. For heel to shin tap instruct the subject to count 8 taps with heel raised about 8 " each time. It is preferable to do this section with the patient lying down. If this is not followed for a particular subject, it should be done in the same position each time. For items in Section C, increments of 0.5 may be used if examiner feels an item falls between two severities. | |
| **1. Heel Along Shin Slide** (Perform while seated or supine, under visual control, slide heel on the contralateral tibia from the patella to the ankle up and down with contralateral leg extended, 3 cycles at moderate speed, one leg at a time.)<br>0 - Normal (stay on shin).<br>1 - Mild (abnormally slow, tremulous but contact maintained).<br>2 - Moderate (goes off shin a total of 3 or fewer times during 3 cycles).<br>3 - Severe (goes off shin 4 or more times during 3 cycles).<br>4 - Too poorly coordinated to attempt the task.<br>**Position of patient: _____** | **Score (Right)** ☐☐.☐<br>**Score (Left)** ☐☐.☐ |
| **2. Heel-to-Shin Tap** (Subject taps heel on midpoint of opposite shin 8 times on each side from about 6-10", one at a time. Perform seated or supine.)<br>0 - Normal (stays on target).<br>1 - Mild (misses shin 2 or <less times).<br>2- Moderate (misses shin 3-5 times).<br>3 - Severe (misses shin greater than times).<br>4 - Too poorly coordinated to perform task.<br>**Position of patient: _____** | **Score (Right)** ☐☐.☐<br>**Score (Left)** ☐☐.☐ |
| **TOTAL LOWER LIMB COORDINATION SCORE** | ☐☐☐.☐ |

**D. PERIPHERAL NERVOUS SYSTEM**

Peripheral nervous system: these items are self-explanatory. Check deltoids and intrinsic hand muscles in the upper limbs, iliopsoas and tibialis anterior in the lower limbs. Atrophy and weakness are scored on the basis of the worst muscle in this group. One does not have to do extensive muscle testing. Vibration sense is recorded as noted in seconds and then given a score depending on the extent of impairment. DTR are recorded in the given space as noted and then any hypo/areflexia is given a numerical score as noted.

**1. Muscle Atrophy** (score most severe atrophy in either upper or lower limb)

0 - None.

1 - Present - mild/moderate

2 - Severe/total wasting

**If atrophy is present or severe, indicate location of atrophy (R):_____**

**If atrophy is present or severe, indicate location of atrophy (L):_____**

**Score (Right)** ☐

**Score (Left)** ☐

**2. Muscle Weakness** (Test deltoids, interossei, iliopsoas and tibialis anterior. Score most severe weakness in either upper or lower limb)

0 - Normal (5/5).

1 - Mild (movement against resistance but not full power 4/5).

2 - Moderate (movement against gravity but not with added resistance 3/5)

3 - Severe (movement of joint but not against gravity 2/5).

4 - Near paralysis (muscular activity without movement 1/5)

5 - Total paralysis (0/5)

**Score (Right)** ☐

**Score (Left)** ☐

**3. Vibratory Sense** (Educate patient regarding the sensation at the elbow. Tested with 128 cps tuning fork set to near full vibration; eyes closed; test over index finger and top of great toe (most distal joint not nail). Abnormal less than 15 seconds for toes and less than 25 seconds for hands)

**3a. Time felt for toes (R): _____**

**3b. Time felt for toes (L): _____**

**3c. Time felt for Fingers (R): _____**

**3d. Time felt for Fingers (L): _____**

| **3e. RIGHT** | **3e. LEFT** |
|---|---|
| 0 - Normal. | 0 - Normal. |
| 1 - Impaired at toes or fingers. | 1 - Impaired at toes or fingers |
| 2 - Impaired at toes and fingers. | 2 - Impaired at toes and fingers. |

**Score (Right)** ☐

**Score (Left)** ☐

| | |
|---|---|
| **4. Position Sense (**test using minimal random movement of distal interphalangeal joints of index finger and big toe**)**<br>0 - Normal.<br>1 - Impaired at toes or fingers.<br>2 - Impaired at toes and fingers. | **Score (Right)** ☐<br>**Score (Left)** ☐ |
| **5. DTR** (0 = absent, 1 = hyporeflexia, 2 = normal, 3 = hyperreflexia, 4 = pathologic hyperreflexia)<br>**5a. RIGHT** **5b. LEFT**<br>Elbow (BJ): _____ Elbow (BJ): _____<br>Wrist (BrJ): _____ Wrist (BrJ): _____<br>Knee (KJ): _____ Knee (KJ): _____<br>Ankle (AJ): _____ Ankle (AJ): _____<br>**5c. RIGHT**<br>0 – No areflexia.<br>1 – Areflexia or mild hyperreflexia in either upper or lower limbs.<br>2 – Generalized areflexia or pathologic hyperreflexia.<br>**5c. LEFT**<br>0 – No areflexia.<br>1 – Areflexia or mild hyperreflexia in either upper or lower limbs.<br>2 – Generalized areflexia or pathologic hyperreflexia. | **Score (Right)** ☐.☐<br>**Score (Left)** ☐.☐ |
| **TOTAL PERIPHERAL NERVOUS SYSTEM SCORE** | ☐☐.☐ |

**E. UPRIGHT STABILITY**

For sitting posture patient can sit in a chair or examination table. For standing and walking assessment instruct patient to wear best walking shoes and record below if barefoot, footwear or AFOs used. Stance assessment begins with feet 20 cm apart. Place marker tapes in the exam room 20 cm apart and the insides of the feet are lined up against these. Subsequent stance tests get more difficult. For feet together the entire inside of the feet should be close together as much as possible. For tandem stance, the dominant foot is in the back and the heel of the other foot is lined with the toes of the dominant foot but not in front of the toes (because this makes it even more difficult). For one-foot stance, the patient is asked to stand on dominant foot and the other leg is elevated by bringing it forward with knee extended; this gives some advantage to the patient. If a patient can stand in a particular position for 1 minute or longer in trial 1, the trials 2 and 3 are abandoned. Otherwise each of 3 trials is timed and then averaged. Grading scores are then given as noted. Tandem walk and gait are performed in a hallway. Preferably no carpet but at least serial examinations should be on the same surface. For gait place markers 25 feet apart. Patient walks the distance turns around and comes back and the activity is timed. Note if the gait was achieved with or without device and serial examinations should be done with the same device as in the first examination.

**Ea. Is subject:**

☐ Barefoot ☐ Footwear

**Eb. Indicate if AFO's (plastic brace) are used?**

☐ No

☐ Yes

**Ec. Test performed on Carpet?**

☐ No

☐ Yes

**1. Sitting Posture** (Subject seated in chair with thighs together, arms folded across chest, back unsupported; observe for 30 sec.)

0 - Normal.

1 - Mild oscillations of head/trunk without touching chair back or side.

2 - Moderate oscillations of head/trunk; needs contact with chair back or side for stability.

3 - Severe oscillations of head/trunk; needs contact with chair back or side for stability.

4 - Support on all 4 sides for stability.

**Score** ☐

| | |
|---|---|
| **2a. Stance - feet apart – Eyes open** (Inside of feet 20 cm apart marked on floor. Use stopwatch; 3 attempts; time in seconds. If greater than 60 seconds on trial 1 stop, if less than 60 seconds do all three trials.)<br>0 - 1 minute or longer. Time 1: _____<br>1 – less than 1 minute, greater than 45 sec.<br>2 – less than 45 sec., greater than 30 sec. Time 2: _____<br>3 – less than 30 sec., greater than 15 sec.<br>4 – less than 15 sec. or needs hands held by assistant/device. Time 3: _____ | **Score 1** ☐<br>**Score 2** ☐<br>**Score 3** ☐<br>**Avg. Score** ☐.☐ |
| **2b. Stance - feet apart – Eyes closed (**If greater than 60 seconds on trial 1 stop, if less than 60 seconds do all three trials.)<br>0 - 1 minute or longer. Time 1: _____<br>1 – less than 1 minute, greater than 45 sec.<br>2 – less than 45 sec., greater than 30 sec. Time 2: _____<br>3 – less than 30 sec., greater than 15 sec.<br>4 – less than 15 sec. or needs hands held by assistant/device. Time 3: _____ | **Score 1** ☐<br>**Score 2** ☐<br>**Score 3** ☐<br>**Avg. Score** ☐.☐ |
| **3a. Stance - feet together** (use stopwatch; 3 attempts; time in seconds. If greater than 60 seconds on trial 1 stop, if less than 60 seconds do all three trials.)<br>0 - 1 minute or longer. Time 1: _____<br>1 – less than 1 minute, greater than 45 sec.<br>2 – less than 45 sec., greater than 30 sec. Time 2: _____<br>3 – less than 30 sec., greater than 15 sec.<br>4 – less than 15 sec. or needs hands held by assistant/device. Time 3: _____ | **Score 1** ☐<br>**Score 2** ☐<br>**Score 3** ☐<br>**Avg. Score** ☐.☐ |

| | |
|---|---|
| **3b. Stance - feet together** – **Eyes Closed (** If greater than 60 seconds on trial 1 stop, if less than 60 seconds do all three trials.)<br>0 - 1 minute or longer. Time 1: _____<br>1 – less than 1 minute, greater than 45 sec.<br>2 – less than 45 sec., greater than 30 sec. Time 2: _____<br>3 – less than 30 sec., greater than 15 sec.<br>4 – less than 15 sec. or needs hands held by assistant/device. Time 3: _____ | **Score 1** ☐<br>**Score 2** ☐<br>**Score 3** ☐<br>**Avg. Score** ☐.☐ |
| **4. Tandem Stance** (dominant foot in front; front foot lined up with great toe of the back foot. If greater than 60 seconds on trial 1 stop, if less than 60 seconds do all three trials.)<br>0 - 1 minute or longer. Time 1: _____<br>1 – less than 1 minute, greater than 45 sec.<br>2 – less than 45 sec., greater than 30 sec. Time 2: _____<br>3 – less than 30 sec., greater than 15 sec.<br>4 – less than 15 sec. or needs hands held by assistant/device. Time 3: _____ | **Score 1** ☐<br>**Score 2** ☐<br>**Score 3** ☐<br>**Avg. Score** ☐.☐ |
| **5. Stance on Dominant Foot** (elevate leg straight out in front, use stopwatch; 3 attempts; time in seconds. If greater than 60 seconds on trial 1 stop, if less than 60 seconds do all three trials.)<br>0 - 1 minute or longer. Time 1: _____<br>1 – less than 1 minute, greater than 45 sec.<br>2 – less than 45 sec., greater than 30 sec. Time 2: _____<br>3 – less than 30 sec., greater than 15 sec.<br>4 – less than 15 sec. or needs hands held by assistant/device. Time 3: _____ | **Score 1** ☐<br>**Score 2** ☐<br>**Score 3** ☐<br>**Avg. Score** ☐.☐ |

| | |
|---|---|
| **6. Tandem Walk** (tandem walk 10 steps in straight line; performed in hallway with no furniture within reach of 1 m / 3 ft. and no loose carpet)<br>0 - Normal (able to tandem walk greater than 8 sequential steps).<br>1 - Able to tandem walk in less than perfect manner/can tandem walk greater than 4 sequential steps, but less than 8.<br>2 - Can tandem walk, but fewer than 4 steps before losing balance.<br>3 - Too poorly coordinated to attempt task. | **Score** ☐ |
| **7. Gait** (observe subject walk at normal pace with assistive device in one direction, turn around and return to start; performed in hallway with no furniture within reach of 1 m / 3 ft. and no loose carpet)<br>0 - Normal.<br>1 - Mild ataxia/veering/difficulty in turning; no cane/other support needed to be safe.<br>2 - Walks with definite ataxia; may need intermittent support/or examiner needs to walk with patient for safety sake.<br>3 - Moderate ataxia/veering/difficulty in turning; walking requires cane/holding onto examiner with one hand to be safe.<br>4 - Severe ataxia/veering; walker or both hands of examiner needed.<br>5 - Cannot walk even with assistance (wheelchair bound). | **Score** ☐ |
| **TOTAL UPRIGHT STABILITY SCORE** | ☐☐.☐ |
| **TOTAL FARS**<br>**(Add subtotals for sections A, B, C, D, & E)** | ☐☐☐.☐ |

# 19.3. Clinician-rated Clinical Global Impression (CGI)

**1. Severity of Illness**

Considering your total clinical experience with this particular population, how ill is the patient at this time

0 = Not assessed
1 = Normal, not at all ill
2 = Borderline ill
3 = Mildly ill
4 = Moderately ill
5 = Markedly ill
6 = Severely ill
7 = Among the most extremely ill patients

**2. Global Improvement**

Rate total improvement whether or not, in your judgement, it is due entirely to drug treatment. Compared to subject's condition at admission to the study, how much has the subject changed?

0 = Not assessed
1 = Very much improved
2 = Much improved
3 = Minimally improved
4 = No change
5 = Minimally worse
6 = Much worse
7 = Very much worse

**3. Efficacy index**

Rate this item on the basis of **drug effect only.**

Select the terms which best describe the degrees of therapeutic effect and side effects and record the number in the box where the two items intersect.

EXAMPLE: Therapeutic effect is rated as 'Moderate' and side effects are judged 'Do not significantly interfere with patient's functioning.'

| **Therapeutic effect** | | **Side Effects** | | | |
|---|---|---|---|---|---|
| | | *None* | *Do not significantly interfere with patient's functioning* | *Significantly interfer with patient's functioning* | *Outweighs therapeutic effect* |
| **Marked** | Vast improvement. Complete or nearly complete remission of all symptoms | 01 | 02 | 03 | 04 |
| **Moderate** | Decided improvement. Partial remission of symptoms | 05 | 06 | 07 | 08 |
| **Minimal** | Slight Improvement which doesn't alter status of care of patient | 09 | 10 | 11 | 12 |
| **Unchanged or worse** | | 13 | 14 | 15 | 16 |

Not assessed = 00

# 19.4. FARS -Activities of Daily Living (FARS-ADL)

**ACTIVITIES OF DAILY LIVING** (increments of 0.5 may be used if strongly felt that a task falls between 2 scores)

1. **Speech** ☐

   0 - Normal
   1 - Mildly affected. No difficulty being understood.
   2 - Moderately affected. Sometimes asked to repeat statements.
   3 - Severely affected. Frequently asked to repeat statements.
   4 - Unintelligible most of the time.

2. **Swallowing** ☐

   0 - Normal.
   1 - Rare choking (< once a month).
   2 - Frequent choking (< once a week, > once a month).
   3 - Requires modified food or chokes multiple times a week. Or patient avoids certain foods.
   4 - Requires NG tube or gastrostomy feedings.

3. **Cutting Food and Handling Utensils** ☐

   0 - Normal.
   1 - Somewhat slow and clumsy, but no help needed.
   2 - Clumsy and slow, but can cut most foods with some help needed. Or needs assistance when in a hurry.
   3 - Food must be cut by someone, but can still feed self slowly.
   4 - Needs to be fed.

4. **Dressing** ☐

   0 - Normal.
   1 - Somewhat slow, but no help needed.
   2 - Occasional assistance with buttoning, getting arms in sleeves, etc. or has to modify activity in some way (e.g. Having to sit to get dressed; use velcro for shoes, stop wearing ties, etc.).
   3 - Considerable help required, but can do some things alone.
   4 - Helpless.

5. **Personal Hygiene** ☐

   0 - Normal.
   1 - Somewhat slow, but no help needed.
   2 - Very slow hygienic care or has need for devices such as special grab bars, tub bench, shower chair, etc.
   3 - Requires personal help with washing, brushing teeth, combing hair or using toilet.
   4 - Fully dependent

**6. Falling (assistive device = score 3)**

0 - Normal.
1 - Rare falling (< once a month).
2 - Occasional falls (once a week to once a month).
3 - Falls multiple times a week or requires device to prevent falls.
4 - Unable to stand or walk.

**7. Walking (assistive device = score 3)**

0 - Normal.
1 - Mild difficulty, perception of imbalance.
2 - Moderate difficulty, but requires little or no assistance.
3 - Severe disturbance of walking, requires assistance or walking aids.
4 - Cannot walk at all even with assistance (wheelchair bound).

**8. Quality of Sitting Position**

0 - Normal.
1 - Slight imbalance of the trunk, but needs no back support.
2 - Unable to sit without back support.
3 - Can sit only with extensive support (Geriatric chair, posy, etc.).
4 - Unable to sit.

**9. Bladder Function (if using drugs for bladder, automatic score of 3)**

0 - Normal.
1 - Mild urinary hesitance, urgency or retention (< once a month).
2 - Moderate hesitance, urgency, rare retention/incontinence (> once a month, but < once a week).
3 - Frequent urinary incontinence (> once a week).
4 - Loss of bladder function requiring intermittent catheterization/indwelling catheter.

**TOTAL ACTIVITIES OF DAILY LIVING SCORE:**

# 19.5. SF-36

**Medical Outcomes Study Questionnaire Short Form 36 Health Survey**

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Thank you for completing this survey! For each of the following questions, please circle the number that best describes your answer.

| **1. In general, would you say your health is:** | |
|---|---|
| Excellent | 1 |
| Very good | 2 |
| Good | 3 |
| Fair | 4 |
| Poor | 5 |
| **2. Compared to one year ago,** | |
| Much better now than one year ago | 1 |
| Somewhat better now than one year ago | 2 |
| About the same | 3 |
| Somewhat worse now than one year ago | 4 |
| Much worse now than one year ago | 5 |

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?
**(Circle One Number on Each Line)**

| | **Yes, Limited a Lot (1)** | **Yes, Limited a Little (2)** | **No, Not limited at All (3)** |
|---|---|---|---|
| a. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports | 1 | 2 | 3 |
| b. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf | 1 | 2 | 3 |
| c. Lifting or carrying groceries | 1 | 2 | 3 |
| d. Climbing **several** flights of stairs | 1 | 2 | 3 |
| e. Climbing **one** flight of stairs | 1 | 2 | 3 |
| f. Bending, kneeling, or stooping | 1 | 2 | 3 |

| | | | |
|---|---|---|---|
| g. Walking **more than a mile** | 1 | 2 | 3 |
| h. Walking **several blocks** | 1 | 2 | 3 |
| i. Walking **one block** | 1 | 2 | 3 |
| j. Bathing or dressing yourself | 1 | 2 | 3 |

4. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?
**(Circle One Number on Each Line)**

| | **Yes (1)** | **No (2)** |
|---|---|---|
| a. Cut down the amount of time you spent on work or other activities | 1 | 2 |
| b. **Accomplished less** than you would like | 1 | 2 |
| c. Were limited in the **kind** of work or other activities | 1 | 2 |
| d. Had **difficulty** performing the work or other activities (for example, it took extra effort) | 1 | 2 |

5. During the **past 4 weeks,** have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?
**(Circle One Number on Each Line)**

| | **Yes** | **No** |
|---|---|---|
| a. Cut down the amount of time you spent on work or other activities | 1 | 2 |
| b. **Accomplished less** than you would like | 1 | 2 |
| c. Didn't do work or other activities as **carefully** as usual | 1 | 2 |

| **6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?** | |
|---|---|
| Not at all | 1 |
| Slightly | 2 |
| Moderately | 3 |
| Quite a bit | 4 |
| Extremely | 5 |

| **7. How much bodily pain have you had during the past 4 weeks?** | |
|---|---|
| None | 1 |
| Very mild | 2 |
| Mild | 3 |
| Moderate | 4 |
| Severe | 5 |
| Very severe | 6 |
| **8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?** | |
| Not at all | 1 |
| A little bit | 2 |
| Moderately | 3 |
| Quite a bit | 4 |
| Extremely | 5 |

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling. **(Circle One Number on Each Line)**

9. How much of the time during the **past 4 weeks** . . .

| | **All of the Time** | **Most of the Time** | **A Good Bit of the Time** | **Some of the Time** | **A Little of the Time** | **None of the Time** |
|---|---|---|---|---|---|---|
| a. Did you feel full of pep? | 1 | 2 | 3 | 4 | 5 | 6 |
| b. Have you been a very nervous person? | 1 | 2 | 3 | 4 | 5 | 6 |
| c. Have you felt so down in the dumps that nothing could cheer you up? | 1 | 2 | 3 | 4 | 5 | 6 |
| d. Have you felt calm and peaceful? | 1 | 2 | 3 | 4 | 5 | 6 |
| e. Did you have a lot of energy? | 1 | 2 | 3 | 4 | 5 | 6 |

| | All of the Time | Most of the Time | A Good Bit of the Time | Some of the Time | A Little of the Time | None of the Time |
|---|---|---|---|---|---|---|
| f. Have you felt downhearted and blue? | 1 | 2 | 3 | 4 | 5 | 6 |
| g. Did you feel worn out? | 1 | 2 | 3 | 4 | 5 | 6 |
| h. Have you been a happy person? | 1 | 2 | 3 | 4 | 5 | 6 |
| i. Did you feel tired? | 1 | 2 | 3 | 4 | 5 | 6 |

| **10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)? (Circle One Number)** | |
|---|---|
| All of the time | 1 |
| Most of the time | 2 |
| Some of the time | 3 |
| A little of the time | 4 |
| None of the time | 5 |

**11. How TRUE or FALSE is each of the following statements for you. (Circle One Number on Each Line)**

| | Definitely True | Mostly True | Don't Know | Mostly False | Definitely False |
|---|---|---|---|---|---|
| a. I seem to get sick a little easier than other people | 1 | 2 | 3 | 4 | 5 |
| b. I am as healthy as anybody I know | 1 | 2 | 3 | 4 | 5 |
| c. I expect my health to get worse | 1 | 2 | 3 | 4 | 5 |
| d. My health is excellent | 1 | 2 | 3 | 4 | 5 |

## 19.6. Patient Global Impression of Change Scale (PGI-C)

**1.** Please indicate how you feel now, compared to how you felt before receiving treatment in this study ***(Choose one)***

1 = Very Much Improved
2 = Much Improved
3 = Minimally Improved
4 = No Change
5 = Minimally Worsened
6 = Much Worsened
7 = Very Much Worsened

## 19.7. Visual Analogue Scale (VAS)

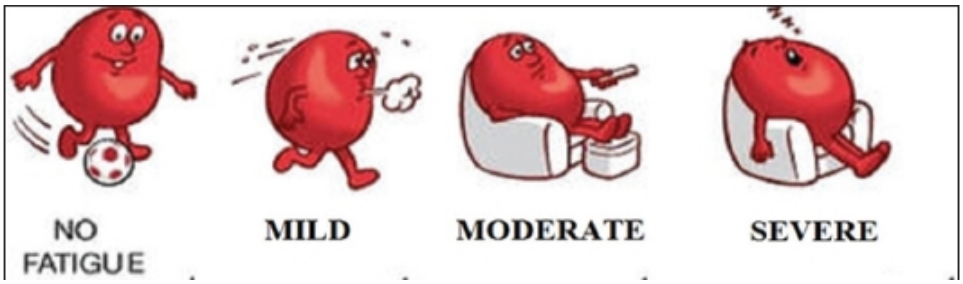
### Visual Analogue Scale for Improvement of FA Symptoms

Rate your Friedreich's Ataxia symptoms compared to baseline (prior to taking study medication) whether or not, in your judgement, it is due entirely to drug treatment. Please circle the appropriate happy/sad face. You can also circle "no change".

0 = Very much improved
2 = Much improved
4 = Minimally improved
6 = Minimally worse
8 = Much worse
10 = Very much worse


### Visual Analogue Scale for Fatigue

How would you rate your fatigue level over the last week?



## 19.8. Fatigue Scale

Below is a list of statements that other people with your illness have said are important. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

| | | Not at all | A little bit | Some-what | Quite a bit | Very much |
|---|---|---|---|---|---|---|
| HI7 | I feel fatigued | 0 | 1 | 2 | 3 | 4 |
| HI12 | I feel weak all over | 0 | 1 | 2 | 3 | 4 |
| An1 | I feel listless ("washed out") | 0 | 1 | 2 | 3 | 4 |
| An2 | I feel tired | 0 | 1 | 2 | 3 | 4 |
| An3 | I have trouble starting things because I am tired | 0 | 1 | 2 | 3 | 4 |
| An4 | I have trouble finishing things because I am tired | 0 | 1 | 2 | 3 | 4 |
| An5 | I have no energy | 0 | 1 | 2 | 3 | 4 |
| An7 | I have trouble doing my usual activities | 0 | 1 | 2 | 3 | 4 |
| An8 | I need to sleep during the day | 0 | 1 | 2 | 3 | 4 |
| An12 | I am too tired to eat | 0 | 1 | 2 | 3 | 4 |
| An14 | I need help doing my usual activities | 0 | 1 | 2 | 3 | 4 |
| An15 | I am frustrated by being too tired to do the things I want to do | 0 | 1 | 2 | 3 | 4 |
| An16 | I have to limit my social activity because I am tired | 0 | 1 | 2 | 3 | 4 |

## 19.9. Neuro-QoL scales

Neuro-QOL Item Bank v1.0 – Lower Extremity Function (Mobility) – Short Form

### Lower Extremity Function (Mobility) – Short Form

**Please respond to each question or statement by marking one box per row.**

| | | Without any difficulty | With a little difficulty | With some difficulty | With much difficulty | Unable to do |
|---|---|---|---|---|---|---|
| NQMOB37 | Are you able to get on and off the toilet?... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQMOB30 | Are you able to step up and down curbs?... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQMOB26 | Are you able to get in and out of a car?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQMOB32 | Are you able to get out of bed into a chair?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQMOB25 | Are you able to push open a heavy door?.. | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQMOB33 | Are you able to run errands and shop?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQMOB31 | Are you able to get up off the floor from lying on your back without help?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQMOB28 | Are you able to go for a walk of at least 15 minutes?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |



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Neuro-QOL Item Bank v1.0 – Upper Extremity Function (Fine Motor, ADL) – Short Form

## Upper Extremity Function (Fine Motor, ADL) – Short Form

**Please respond to each question or statement by marking one box per row.**

| | | Without any difficulty | With a little difficulty | With some difficulty | With much difficulty | Unable to do |
|---|---|---|---|---|---|---|
| NQUEX29 | Are you able to turn a key in a lock?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQUEX26 | Are you able to brush your teeth?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQUEX44 | Are you able to make a phone call using a touch tone key-pad?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQUEX36 | Are you able to pick up coins from a table top?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQUEX30 | Are you able to write with a pen or pencil?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQUEX28 | Are you able to open and close a zipper?... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQUEX33 | Are you able to wash and dry your body?.. | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| NQUEX37 | Are you able to shampoo your hair?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |



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## 19.10. Fall Questionnaire

1. How many times in the last year have you:

Fallen to the ground: _____

Had near falls (this means you lose your balance but retain your upright position, for instance, by grabbing on to something): _____

_____

Stumble and catch yourself: _____

2. Did you ever hurt yourself because of a fall?

- ○ No
- ○ Once
- ○ More than once

3. What kind of injuries have you suffered as a consequence of a fall?

- ○ None
- ○ A fracture (i.e. A hip): _____
- ○ Other wounds (i.e. Bruises, scrapes, cuts): _____
- ○ Loss of consciousness
- ○ Other (i.e. Internal bleeding, including the brain): _____

4. When you fall, where does this usually take place?

- ○ Outdoors
- ○ Indoors
- ○ Both

5. When you fall, at what time does this usually take place?

- ○ Any time of day
- ○ Daytime
- ○ Evening
- ○ Night time (after going to bed)

6. In which direction do you fall most often?

- ○ Backwards
- ○ Forwards
- ○ Sideward right / left (circle the correct answer)
- ○ All directions equally

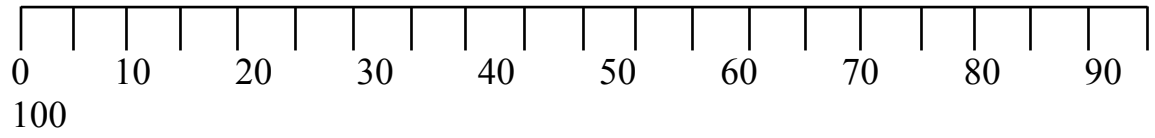
7. What do you do to prevent falling?

- ○ Nothing
- ○ Avoid certain activities (give examples): _____
- ○ The use of aids (i.e. cane, walker, holding on to a person, holding on to furniture): _____
- ○ Other: _____

8. Do you experience a fear of falling?

- ○ No
- ○ Slightly
- ○ Very much

9. Can you give an indication of your confidence in balance during daily activities on a scale of 0 up to 100 on the below mentioned scale? Zero means no confidence and 100 means full confidence in performing the activities?



## 19.11. Speech Assessment Questionnaire

For subject:

**How would you rate the overall quality of your speech?**

| | | | | |
|---|---|---|---|---|
| Very Good | Good | Fair | Poor | Very Poor |

**How would you rate your verbal communication with your family or friends?**

| | | | | |
|---|---|---|---|---|
| Very Good | Good | Fair | Poor | Very Poor |

*For parents, caregiver or travel companion response* ***(not for patients to respond)***
***Enter NA, if not present:***

**How would you rate the subject's overall quality of verbal communication?**

| | | | | |
|---|---|---|---|---|
| Very Good | Good | Fair | Poor | Very Poor |

# 19.12. Optional PK Profile Sub-Study: Pharmacokinetic Profile of RT001 at Steady State in Subjects with Friedreich's Ataxia Who Participated in Study RT001-006

## 19.12.1. Study Objectives

The objective of the study is to evaluate the pharmacokinetic (PK) profile of RT001 at steady state.

## 19.12.2. Study Design

At selected sites, subjects who are enrolled in RT001-006 will be invited to participate in this study. At the time of the Month 11 visit in RT001-006, PK samples will be obtained 30 minutes to 1 hour before the morning dose; and 30 min, 1, 2, 4, 8, 12 and 24 hours after the initial morning dose. It is recommended that the blood draws are done on a different day than the study procedures for the Month 11 visit in RT001-006.

## 19.12.3. Randomization

There will be no randomization. All subjects who are volunteering for this sub-study will be enrolled.

## 19.12.4. Blinding

Subjects and study personnel, including but not limited to investigators, study coordinators, nursing staff, clinical monitors, the Sponsor, and the Sponsor's representatives, will remain blinded to the study treatment assignments (RT001 or placebo) in RT001-006.

## 19.12.5. Inclusion Criteria

1. At selected sites, subjects who are enrolled in RT001-006 will be invited to participate in this study.
2. All participating subjects will be required to sign an informed consent prior to the collection of the blood draws.
3. Subjects must be willing to stay overnight close to the study site and able to provide the necessary repeated blood samples.

## 19.12.6. Sample Size

Subjects who are enrolled in RT001-006 will be invited to participate in this study. Because the study treatment (active or placebo) in RT001-006 is blinded, a minimum of 10 subjects will be enrolled to ensure that at least 5 subjects who are randomized to active are enrolled in this study. An unblinded person may be asked to verify that at least 5 of the selected subjects were randomized to active. Additional subjects may be added as needed.

### 19.12.7. Sample Collection

Bioanalytical measurements will be performed to determine the concentration profiles of D2-LA, D2-ARA, H2-LA and H2-ARA in plasma and red blood cells. Blood samples will be collected according to the schedule below:

Day 1: Hour -1.0 to -0.5 (pre-breakfast, pre-dose), 0.5 (± 5 min), 1 (± 5 min), 2 (± 10 min), 4 (± 15 min) (pre-lunch), 8 (± 15 min) (pre-dinner), and 12 (± 60 min) (post-dinner)

Day 2: 24 hours following dosing on Day 1 (± 60 min; pre-breakfast, pre-dose)

Actual collection time points should be recorded and will be used for PK analysis. Best efforts to collect the samples at the planned time should be made.

Protocol-specific instructions will be provided for the collection, handling, storage, and shipping of PK (plasma and RBC) samples. The date and actual time each sample is collected will be recorded. Date and time of the morning dose will also be recorded. The subject will only take the morning dose on the PK collection day.

### 19.12.8. Timing of Meals and Study Drug

The timing of the meals and study drug needs to be planned in relationship to the timing of the sample collection. The subjects will need to consume an in-clinic standardized meal (breakfast) which will be administered at Hour -0.5 and should be consumed within 30 minutes. Patients will need to take the morning dose in the clinic, 30 minutes after the start of their breakfast, at Hour 0.

Lunch can be eaten after the 4-hour sample is collected. Dinner can be eaten after the 8-hour sample is collected, but before the 12-hour sample is collected. The subject will only take the morning dose on the PK collection day.

### 19.12.9. Data Analysis

Bioanalytical measurements will be performed to determine plasma concentration profiles of D2-LA, D2-ARA, H2-LA, and H2-ARA. PK parameters will include area under the concentration-time curve (AUC), maximum observed plasma concentration ($C_{max}$), and time to reach maximum plasma concentration ($T_{max}$). Full details of planned analyses will be provided in a separate PK analysis plan.

# 19.13. Optional Washout Sub-Study: Washout Blood Levels of Subjects Who Have Completed Treatment in RT001-006

## 19.13.1. Study Objectives

The objective of the study is to evaluate the washout blood levels of RT001 after treatment in RT001-006 is completed.

## 19.13.2. Study Design

Subjects who completed treatment in RT001-006 will be invited to participate in this sub-study. Subjects who complete RT001-006 are eligible to participate in any part of the PK sub-study. For example, a subject may enroll 4 months after completing the treatment period of RT001-006. For such a subject, only the Month 6 PK washout sample will be obtained and analyzed. Depending on the timing of entry into the sub-study, washout pharmacokinetic (PK) samples will be obtained at 7 days, 4, 12, and 24 weeks after the last dose to determine the trough concentration of deuterated linoleic acid (D2-LA), deuterated arachidonic acid (D2-ARA), nondeuterated linoleic acid (H2-LA) and nondeuterated arachidonic acid (H2-ARA) in plasma, and D2-LA, D2-ARA H2-LA, and H2-ARA in red blood cells.

The samples may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.

## 19.13.3. Randomization

There will be no randomization. All subjects who volunteer for this sub-study will be enrolled.

## 19.13.4. Blinding

Subjects and study personnel, including but not limited to investigators, study coordinators, nursing staff, clinical monitors, the Sponsor, and the Sponsor's representatives, will remain blinded to the study treatment assignments (RT001 or placebo) in RT001-006.

## 19.13.5. Inclusion Criteria

1. Subjects who have successfully completed treatment in RT001-006 will be invited to participate in the washout PK sub-study.
2. All participating subjects will be required to sign an informed consent prior to any blood draws.
3. Subjects must be willing and able to provide the necessary repeated blood samples.

## 19.13.6. Sample Size

Subjects who have successfully completed the treatment period in the RT001-006 trial and meet all entry criteria will be invited to participate in this sub-study.

## 19.13.7. Sample Collection

Bioanalytical measurements will be performed to determine the concentration of D2-LA, D2-ARA, H2-LA and H2-ARA in plasma and red blood cells. Blood samples will be collected for

plasma measurements and assessment in red blood cells at 7 days (± 1 day), at 4 weeks (± 7 days), at 12 weeks (± 7 days) and at 24 weeks (± 7 days). Collection time points should be recorded and will be used for PK analysis. Best efforts to collect the samples at the planned time should be made. The sample may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.

Instructions will be provided for the collection, handling, storage, and shipping of PK (plasma and RBC) samples in a separate document. The date and actual time each sample is collected will be recorded.

### 19.13.8. Data Analysis

The PK of D2-LA, D2-ARA, H2-LA, and H2-ARA will be characterized in terms of minimum and maximum levels after washout. Full details of planned analyses will be provided in a separate PK analysis plan.