A Phase II, Open Label Prospective Single Center Drug Study Evaluating the Safety and Efficacy of (+)-Epicatechin in Subjects with Friedreich's Ataxi

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Study Product: (+) Epicatechin capsules

Protocol Number: 15-006845

IND Number: 128799

Version No.	Date	Major Changes
1	18Nov15	Initial
2	25Mar16	IND # added; Section 4.5 added: replacement of dropped/withdrawn subjects; Vitals/BP added to study visits; Platelets and liver enzyme panel added to lab tests; Protocol Schedule of Events updated
3	18 Oct 16	Removed PaTa test from FARS test
4	17 Dec 16	Section 3 added Cerebral MRI is on 3T scanner Add pregnancy test week 24 Only Troponin I will be used because using both Troponin I and T is redundant Footnote i exploratory test made optional if budget allows Protocol SOE updated
5	27 Jan 17	Remove glucose tolerance test from week 12 Change FARS scale improvement to a decrease of greater than 1 in section 3.1 Section 5.8.2 changed to reflect new information about keeping the drug refrigerated.

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List of Abbreviations

AKT	Protein kinase B
ALT/AST	Alanine Aminotransferase/Aspartate Aminotransferase
AMPK	AMP-activated kinase
BNP	B-Type Natriuretic Peptide
BOLD	Blood Oxygenation Level Dependent
CFR	Code of Federal Regulations
Cho	Choline
Cr	Creatine
CRF	Case Report Form
CYP	Cytochrome P450
DTI	Diffusion Tensor Imaging
EPI	Epicatechin
ETC	Electron Transport Chain
FA	Friedreich's Ataxia
FARA	Friedreich's Ataxia Research Alliance
FARS	Friedreich's Ataxia Rating Scale
FDA	Food and Drug Administration
Fe-S	Iron-Sulfur
GCP	Good Clinical Practice Guidelines
HERG	Human Ether-a-Go-go Related Gene
IND	Investigational New Drug (application)
LAR	Legally Authorized Representative
LKB	Liver Kinase B1
LV	Left Ventricular
Mg	Milligrams
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MtD	Mitochondrial Disease
Myf5	Myogenic Factor 5
MyoD	Myogenic Differentiation Antigen
NAA	N-acetyl Aspartate
NAMDC	North American Mitochondrial Disease Consortium
NO	Nitric oxide
Nrf2	nuclear factor-E2-related factor-2
OHP	Hydroxy-pregnenolone
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K	Phosphoinositide 3-kinase
PT/PTT	Prothrombin Time/Partial Prothrombin Time
ROS	Reactive Oxygen Species
SIRT3	Sirtuin 3
SOD2	Superoxide dismutase 2
ST2	Suppressor of tumorigenicity 2
TID	Ter in die (three times a day)

Study Summary

Title	A Phase II, Open Label Prospective Single Center Drug Study Evaluating the Safety and Efficacy of (+)-Epicatechin in Subjects with Friedreich's Ataxia
Running Title	Safety and Efficacy of (+)-Epicatechin in Friedreich's Ataxia
Protocol Number	15-006845
Phase	Phase II
Methodology	This is an open label, single center, prospective phase II drug trial to assess the safety and efficacy of (+)-EPI in the treatment of Friedreich's Ataxia. Subjects will be evaluated at outpatient clinic visit appointments and interested qualified subjects will be consented and offered participation in this trial. Once consent has been obtained, baseline values will be established and subjects will begin treatment and follow-up over a 24 week period. Clinical and biochemical parameters will be assessed at baseline and at 12 and 24 weeks after initiation of treatment.
Overall Study Duration	Two years estimated to complete enrollment
Subject Participation Duration	24 weeks
Single or Multi-Site	Single center
Objectives	Primary: To assess the safety and clinical efficacy from neurological, cardiac and metabolic evaluations of patients before and after treatment. Secondary: To assess (i) up-regulation of relevant mitochondrial proteins (frataxin and ETC complexes I-V) and (ii) reduction of urine F2-isoprostane, before and after treatment. Exploratory: To assess myocardial oxygenation by Blood Oxygenation Level Dependent (BOLD) sequence cardiac MRI, and myocardial perfusion index by Regadenoson-stress-perfusion cardiac MRI, before and after treatment as non-invasive, more sensitive endpoints for future studies. To investigate neuroimaging biomarkers of disease progression including spinal cord and cerebellar volumetry, Diffusion Tensor Imaging (DTI), and Magnetic Resonance Spectroscopy (MRS).
Number of Subjects	10 subjects

Diagnosis and Main Inclusion Criteria	Study participants between the ages of ≥10 and ≤50 must have a confirmed diagnosis of Friedreich's Ataxia with disease duration ≤7 years and at least one affected organ system (cardiac or neurological)
Study Product, Dose, Route, Regimen	(+)-Epicatechin 25mg capsules will be administered orally TID for 24 weeks, with dose escalation to 50mg TID at 3 months for subjects not showing improvement.
Duration of Administration	24 weeks
Reference therapy	No reference therapy
Statistical Methodology	With a sample size of 10 patients we should have 80% power to detect an effect size of 1 standard deviations or larger on outcome measures

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with this protocol and the applicable United States government regulations including Food and Drug Regulations 21 CFR Part 50 (Human Subject Protections), Part 54 (Financial Disclosure of Clinical Investigators), Part 56 (Institutional Review Boards), Part 312 (IND) and Good Clinical Practice guidelines, as well as the applicable Mayo Clinic research policies and procedures.

1.1 Background

Mitochondrial disease (MtD) includes a large number of human disorders with a collective prevalence of 10-15 cases per 100,000, resulting from functional defects in the mitochondrial electron transport chain (ETC). ETC defects impair the production of ATP and also enhance the generation of reactive oxygen species (ROS), leading to the combination of energy deficit and oxidative damage underlying the pathogenesis of MtD. Given the vital role played by the ETC in mammalian cells, ETC defects may affect virtually any tissue or organ system and present with a wide variety of symptoms in both children and adults. Progressive multisystem involvement occurs in most patients with variable combinations of neurological, muscular, cardiac, metabolic and other deficits [1]. The clinical complexity of MtD is associated with equally significant genetic complexity owing to the fact that a large number of both mitochondrial and nuclear genes are required for the biogenesis and function of the ETC. Indeed, while ETC defects were originally linked to mutations in mitochondrial genes, over 30 nuclear genes were subsequently implicated and more are being identified. Mutations in nuclear genes can cause MtD via a broad spectrum of molecular mechanisms that may affect the integrity of the mitochondrial genome, the assembly of specific ETC complexes, and the synthesis or delivery of metal or Fe-S cofactors needed for ETC activity [1].

Research Idea: While many recent advancements in genetic and biochemical testing facilitate the diagnosis of MtD, there is no effective therapy available for this group of disorders as a whole [2]. In light of the common underlying pathogenic mechanism of energy deficit and oxidative damage, we hypothesize that the induction of mitochondrial biogenesis and bioenergetics along with simultaneous up-regulation of the cellular antioxidant defenses is a strategy capable to ameliorate MtD. To test this hypothesis we propose to conduct a phase II drug clinical trial to test the efficacy of a pharmaceutical grade synthetically-produced form of Epicatechin (EPI) in patients with Friedreich's Ataxia.

Why EPI? (-) EPI is a naturally occurring flavonoid that has shown to have similar health benefits to exercise training [3]. Preliminary observations suggest the existence of a signaling mechanism that may induce mitochondrial biogenesis and lead to regeneration of skeletal muscle fibers and neurons depleted of mitochondria, induction of endogenous anti-oxidant enzymes such as superoxide dismutase and catalase, and stimulation of lipid oxidative metabolism and glucose uptake thereby improving plasma triglycerides and glucose (Figure 1) [[4] and Cardero Therapeutics' unpublished data]. The working hypothesis is that

Figure 1

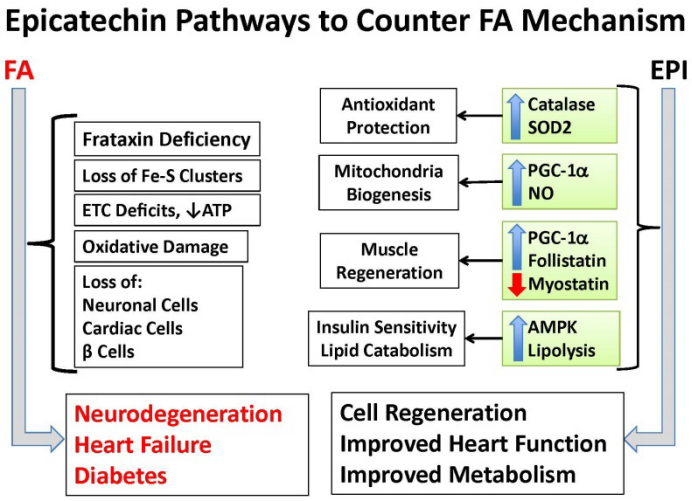


Figure 1: SOD2: Superoxide dismutase 2; PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; NO: Nitric oxide; AMPK: AMP-activated kinase.

this signaling mechanism is physiologically activated during exercise and would activate transcription factors that would induce the effects described above. Therefore, EPI should ameliorate MtD. This working hypothesis is supported by (i) evidence that EPI acts as an exercise mimetic [3]; (ii) the positive effects on cardiovascular disease and type 2 diabetes associated with the consumption of EPI-rich foods [5, 6]; and (iii) the positive effects of purified EPI on mitochondrial biogenesis [7], oxidative stress [8], metabolism [9, 10], skeletal muscle growth and differentiation [11] and neuroprotection [12].

The active compound (-) EPI is found in cocoa powder, green tea and other plants [13]. Recent research has indicated that its enantiomer, (+)-EPI is more potent and more efficacious in vitro and in vivo compared to (-)-EPI with respect to stimulating new mitochondrial formation. (+)-EPI is also a natural product, found in the guarana berry that serves as the source of widely consumed beverages in South America [14]. Guarana is generally recognized as safe and a permitted food additive by FDA [15]. (+)-EPI is not commercially available in current Good Manufacturing Practices (cGMP) grade monomeric form, for any purpose, research or otherwise. Cardero Therapeutics has developed the first scalable manufacturing process that provides pharmaceutical grade (+)-EPI. This manufacturing process has already been accepted by the FDA in the context of a previously filed IND for determining the tolerability and PK profile of (+)-EPI in healthy human volunteers. Given the severity of FA, we propose studying the effects of the more potent enantiomer of epicatechin. In addition, EPI can be administered orally, readily crosses the blood-brain barrier, and is generally recognized as a safe compound [9, 12].

Why FA? FA is a paradigm for MtD in which intronic trinucleotide repeat expansions in the FA gene lead to depletion of the mitochondrial protein frataxin [16]. Frataxin is an essential iron chaperone required for Fe-S cluster assembly, and a reduction in the levels of frataxin leads to ETC dysfunction, energy deficit and oxidative damage via iron-catalyzed Fenton chemistry [17]. FA patients develop progressive neuro-degenerative disease often associated with cardiomyopathy, diabetes mellitus, and muscle weakness with a median age of onset of 10 years. Thus, we believe EPI is a promising compound for the treatment of FA given its unique biological effects that appear likely to be able to counteract the pathophysiology of FA and thereby slow down or stop disease progression.

Therefore, the ability of Epicatechin to reverse oxidative injury, ameliorate congestive heart failure, and induce mitochondrial repletion, coupled with its safety as a dietary product, argues for investigating its therapeutic qualities in patients with FA. Moreover, Cardero Therapeutics have

teamed-up with our multi-disciplinary FA group of clinicians and scientists who share a long interest in MtD and FA at Mayo Clinic, enabling this initial pilot study and future larger studies.

1.2 Investigational Agent

Description:
Cardero Therapeutics has synthesized pharmaceutical grade (+) Epicatechin, a more active natural product enantiomer of epicatechin, which is otherwise not available in a purified form. EPI is associated with signaling mechanism that induces mitochondrial biogenesis via activation of the transcription factor PGC-1α [9], upregulates endogenous antioxidant mechanisms (SOD2, catalase) controlled by the Nrf2 (nuclear factor-E2-related factor-2) pathway [18], promotes fatty acid oxidation through regulation of SIRT3 (Sirtuin 3) signaling [9, 19] and enhances insulin sensitivity through the PI3K/AKT (Phosphoinositide 3-kinase/ Protein kinase B) pathway and AMPK [10] which may further improve metabolic dysfunction in FA.

Pharmacology:
Pharmacokinetics of both isoforms of EPI have been described in a previous IND filed by Dr. Robert Henry (IND 122158), by Barnett et al, 2015, and Ottaviani et al, 2011 [20, 21]. Numerous publications indicate the polyphenol epicatechin does not undergo Phase I hepatic metabolism mediated by cytochrome P450 oxidases. In the liver, (-)-EPI undergoes modification (by uridine-5-diphosphate glucuronosyl-transferases, sulfo-transferases, and catechon-O-methyltransferases), which results in glucuronide, sulfate, or methyl conjugates/metabolites, accounting for more than 85% of the absorbed epicatechin. Tmax ranges from 3.2-3.8 hours and T1/2 ranges from 1.8.-3.8 hours. Virtually no unconjugated epicatechin is observed after 1 hour. Bioavailability is approximately 30%. (+)-EPI demonstrates moderately less bioavailability and less conjugation with glucuronide or sulfate compared to (-)- EPI [21, 22]. It is less effective as an inducer of NO synthesis in vitro and in vivo. The half-life is less than 90 minutes for both enantiomers [21]. In addition, (+)-EPI has no inhibitory effect on the five most important P450 oxidases in a hepatic microsome assay [23]. In vitro, (+)-Epicatechin demonstrates, no Human Ether-a-Go-go Related Gene (HERG) activity [24]and no non-selective interactions with a diverse panel of receptors and enzymes [25].

1.3 Preclinical Data

Effect of (-)-EPI on mitochondrial biogenesis in skeletal muscle and in patients with Diabetes and heart failure. Emerging rodent and human data confirm that (-)-EPI increases mitochondrial biogenesis and the expression of mitochondrial electron transport chain complex proteins by up regulating AMPK, SIRT1 and PGC1-alpha [3, 6, 11, 20, 26-28]. Thus (-)-EPI stimulates muscle mitochondrial mass/volume and increases maximal mitochondrial respiratory rate. In addition, (-)-EPI promotes the ability of muscle to regenerate by upregulating muscle growth promoters (optimizing follistatin/myostatin ratio) [6]. These changes have been documented in the skeletal muscle (SkM), heart, and liver and are accompanied by improvements in exercise performance and congestive heart failure.

(-)-EPI ameliorates oxidative stress and improves cardiac function. In a mouse model (-)-EPI treatment resulted in improved oxidative stress evidenced by recovery of key elements of redox

balance (glutathione/oxidized glutathione ratio, thioredoxin) and enhanced endogenous anti-oxidant enzymes (superoxide dismutase 2, catalase and citrate synthase) [5]. Furthermore, the investigators found decreases in heart and skeletal muscle fibrosis, accompanied by an improvement in muscle function. In a rat model of non-dystrophic heart failure oral administration of (-)-EPI effectively suppressed abdominal aorta ligation-induced CHF [29]. In addition, (-)-EPI demonstrated preservation of myocardial structure and function in a model of chronic heart failure [30].

EPI appears to act as an exercise mimetic [31], based on its positive effects on cardiovascular disease and type 2 diabetes associated with the consumption of EPI-rich foods[13]; and the positive effects of purified EPI on mitochondrial biogenesis and oxidative stress [27], metabolism [13], and skeletal muscle growth and differentiation [32].

EPI is a neuroprotectant and inducer of neural regeneration. Neuronal injury as a result of mitochondrial dysfunction is a component of the pathophysiology of Friedreich's ataxia. There is evidence for direct effects of epicatechin in preserving neurons. Wild-type mice pretreated orally with epicatechin before middle cerebral artery occlusion (MCAO) had significantly smaller brain infarcts and improved neurologic deficit scores (NDS) than did a vehicle-treated group [33].

In a medium throughput screen for neuroprotective compounds using primary mixed neuronal cells and a mitochondrial toxin, epicatechin was discovered to be a potent direct neuroprotectant. It effectively decreased intracellular oxidative stress and normalized the signaling pathway of brain-derived neurotrophic factor (BDNF) [12].

Cell viability assays with neuronal cultures further confirmed that epicatechin can directly protect neurons against oxidative insults. With particular relevance to FA, much of the neuronal benefit appeared to be due to the induction of endogenous anti-oxidant mechanisms controlled by the Nrf2 pathway [18].

Effects of (+)-Epicatechin in Normal Mice. Studies have been performed to evaluate effects on exercise capacity using wild type mice treated with either water (control), (-)-epicatechin (1 mg/kg/day) or (+)-epicatechin (0.1, 0.3 or 1 mg/kg/day). Endpoints evaluated include the mitochondrial biogenesis transcription factors AMPK and PGC1-α. (Figure 2) Results indicate that following 2 weeks of treatment, (+)-epicatechin could enhance exercise capacity (as assessed by treadmill testing, (Figure 3) in a magnitude similar to 1 mg/kg/day of (-)-epicatechin but at lower doses (equivalency noted at 0.3 mg/kg/day). Biochemical analyses (Western blots) of muscle samples from the above mice demonstrated a similar pattern of responses, whereby (+)-epicatechin was as effective at lower doses or more effective at comparable doses when probing for effects on recognized key modulators of metabolism.

Figure 2

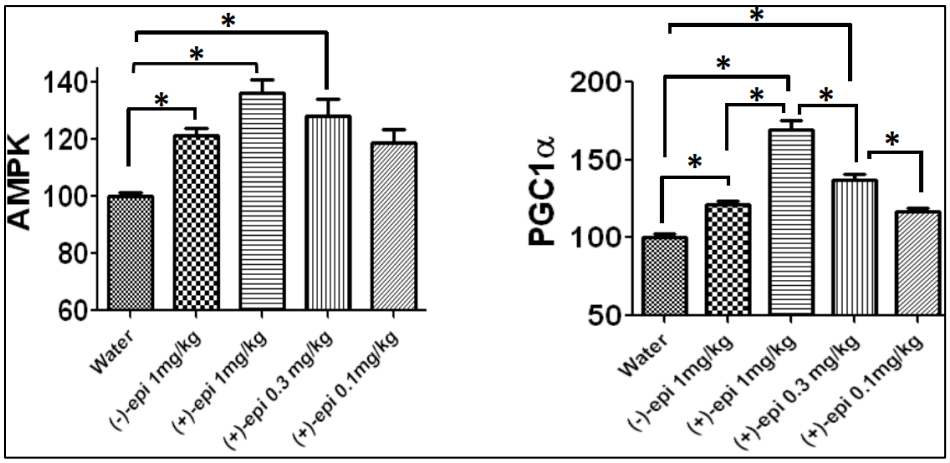
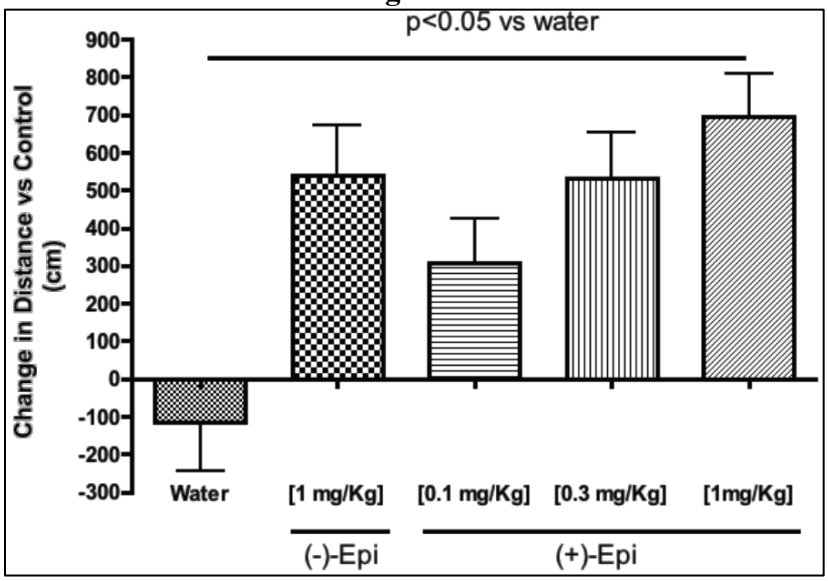


Figure 3



1.4 Clinical Data to Date

Published data for the (-) Epi study results are pertinent to this study, because (+)- EPI is an enantiomer of (-)-EPI with similar biological effects.

Effect of (-)-EPI on mitochondrial biogenesis. Taub and colleagues [6, 28] recently published the results of a study performed in heart failure and DM2 patients in which (-)-EPI-rich cocoa administration restored SkM mitochondrial cristae density and improved the overall mitochondrial function in an orchestrated manner with increased respiratory chain protein activity as well as levels of multiple indicators of mitochondria biogenesis.

Effect of (-)-EPI in Becker Muscular Dystrophy. Dr. Craig McDonald at UC-Davis recently presented the results of a pilot trial in which 7 patients with Becker muscular dystrophy completed an 8 week treatment with (-)-EPI given at 50 mg twice a day [4]. Muscle biopsies at baseline and at

8 weeks demonstrated statistically significant increases in biomarkers of mitochondrial biogenesis (mitofillin and cristae density by EM), hormone and tissue markers of muscle regeneration (follistatin, Myogenic Differentiation Antigen MyoD, Myogenic Factor 5 Myf5, myogenin), enhanced expression of exercise-sensitive muscle transcription factors and muscle contractile proteins (Liver Kinase B1 LKB1, actin and myosin), mitochondrial biogenesis transcription factors (AMPK, PGC1α), and increased levels of muscle contractile proteins [dystrophin, dysferlin, and utrophin]. McDonald also observed an increase in cardiac stroke volume, indicating improved cardiac function. This was confirmed by the finding that within 8 weeks there was a significant decrease in BNP levels per patient, indicating an improvement in the severity of heart failure. Cardero-supplied cGMP grade (+)-epicatechin has been undergoing evaluation at University of California-San Diego in single and multiple dose strengths and frequency, with no adverse events experience thus far.

In addition, when given to prehypertensive adults for 4 weeks at 100 mg/day, (-)-Epicatechin improved insulin resistance [34].

1.5 Dose Rationale and Risk/Benefits

For this study, (+)-EPI will be administered orally at a starting dose of 75 mg total daily dose (25 mg TID) escalated to 150mg total daily dose (50 mg TID) for those subjects not showing improvement at 12 weeks. (+)-EPI plasma levels will be measured before and after 3 (+/-1), 12 (+/-1), and 24 (+/-1) weeks of treatment to assess study drug dosing compliance.
The dosage is supported by 3 factors:

1) Human efficacy has been demonstrated with (-) Epicatechin at 1.0-1.5 mg/kg [4, 20, 34] given at 50 mg twice a day for up to 8 weeks. The twice a day dosing was chosen because once a day dosing did not demonstrate efficacy, presumably because of the short half-life (Barnett et al,2015)
2) At 1 mg/kg in animals, (+)-Epicatechin is more effective in stimulating exercise endurance and activating muscle AMPK and PGC1alpha than (-) –Epicatechin. (Figure 2, Figure 3)
3) Because epicatechin is not subject to Phase 1 hepatic metabolism, the pharmacokinetics in rats is similar to that of humans and the effective dosage in rodents predicts effective dosage in humans [4, 26]. FA patients will range between 25-70 kg in weight. Therefore, we will be well within the in vivo documented range of efficacy in rodent models and within the safe administration of (+)-EPI in previously published pharmacokinetic studies [21].

2 Study Objectives

See Section 3 for detailed descriptions of outcomes measures/endpoints.

Primary Objective(s):
To assess the clinical safety and efficacy of (+)-EPI in the treatment of Friedreich's Ataxia, based on changes from baseline in neurological and cardiac function, following dosing with (+)- EPI.

Secondary Objective(s):
To assess changes from baseline following treatment with (+)-EPI.

- Metabolic status relating to diabetes

- Mitochondrial function

Exploratory Objective(s):
To assess changes from baseline following treatment with (+)-EPI in:

- Myocardial oxygenation and myocardial perfusion, as possible non-invasive, more sensitive endpoints for future studies
- Muscle regeneration as measured by (serum) biomarkers follistatin and myostatin

3 Study Design and Visit Description

See also the Protocol Schedule of Events in Appendix II.

Visit 1: Baseline- Week 0
At the baseline visit, the study team will review participant eligibility. For participants who meet inclusion criteria, an informed consent discussion will take place with subject and LAR, when applicable. Adequate time will be provided for the subject/LAR to read the consent and have any and all questions answered prior to signing the consent form. Study staff will complete a review of medical history and concomitant medications, conduct a physical exam and including neurological assessment with a FARS scoring for enrolled subjects. A 3 month supply of (+)- EPI will be dispensed to the subjects/LAR along with a dosing/adverse event diary to be completed by the subject and/or LAR. The diary entries will be reviewed with participants during subsequent clinic visits. All study tests will be conducted at Mayo Clinic, with the exception of study drug levels and follistatin/myostatin analysis, to be conducted by a research laboratory at UC-San Diego. The following evaluations will be completed:

- Physical Examination, Vitals, Blood Pressure
- Drug level analysis of (+)- EPI in blood
- Follistatin/myostatin analysis in blood
- Friedreich's Ataxia Rating Scale (FARS) Assessment (See Attachment I)
- Urine analysis
    - F2-isoprostane (8-iso prostaglandin F2, an eicosanoid produced by oxidation of tissue phospholipids) to assess for oxidative stress
- Blood chemistry
    - Serum BNP, calcium, cardiac troponin I, CBC, creatinine with eGFR, frataxin, glucose tolerance test, hemoglobin A1C, iron, mitochondrial complex I-V levels, potassium, PT/PTT, sodium, ST2, triglycerides, ALT, AST, Alkaline Phosphatase and ceruloplasmin [35].
- Neurology studies
    - Cerebellar and spinal cord MRI with volumetry, DTI, and MRS analysis
- Echocardiogram and electrocardiogram
- Cardiac MRI with BOLD/Perfusion analysis
- Urine pregnancy test for female subjects
- We may collect blood for off study evaluation if budget allows

- Respiratory chain function, aconitase, catalase, PGC1α, SOD2 in blood

Week 3 (+/- 1 week): Venipuncture- No onsite visit to Mayo Clinic required
A test kit will be mailed or provided at visit 1 by to the participant for venipuncture blood collection by a local physician. The sample shipment will be pre-addressed to the laboratory at UC-San Diego. Test results will subsequently be provided to the PI and maintained with study documents for:

- Drug level analysis of (+)- EPI in blood
- Follistatin/myostatin level in blood

Telephone calls between baseline and 3 month on-site visits: The subjects/LAR will be contacted by study staff approximately every 15 days to assess progress, symptoms and dosing compliance. If it appears subject is experiencing adverse changes and the Investigator feels an on-site visit is warranted, the subject will be asked to come in for an interim clinic visit.

Visit 2: Week 12 (+/- 1 week)
At Visit 2, the participant will undergo a physical exam and FARS scoring. Medical history, changes to concurrent medications, adverse event diary and dosing will be reviewed and additional diaries dispensed, as needed. A physical examination will be conducted. Dosing of (+)- EPI will be adjusted depending on the performance of the 8m timed walk completed through the FARS assessment and/or cardiac assessment via MRI. If the participant shows improvement (as defined in section 3.1) compared to baseline in composite score, including the 8m timed walk or cardiac function by MRI, the medication dosage will remain the same. If the participant shows no improvement or worsening compared to baseline, the (+)- EPI dose will be increased. Additional investigations include:

- Physical Examination, Vitals, Blood Pressure
- Drug level analysis of (+)- EPI in blood
- FARS Assessment
- Blood chemistry
    - Serum BNP, calcium, cardiac troponin I, CBC, ceruloplasmin, creatinine with eGFR, frataxin, , hemoglobin A1C, iron, mitochondrial complex I-V, potassium, PT/PTT, sodium, and ST2, triglycerides, and liver panel (ALT, AST, and Alkaline Phosphatase)
- Cardiac MRI with BOLD
- Urine pregnancy test

Telephone calls between 3 month and 6 month on-site visits: The subjects/LAR will be contacted by study staff approximately every 15 days to assess progress, symptoms and dosing compliance. If it appears subject is experiencing adverse changes and the Investigator feels an on-site visit is warranted, the subject will be asked to come in for an interim clinic visit.

Visit 3: Final Evaluation- Week 24 (+/- 1 week)
At the final visit, the participant will undergo a physical exam and neurological/cardiac assessments. Changes in symptoms and any concurrent medications will be assessed and the dosing/adverse event diary will be reviewed and collected/retained. Final investigations include:

- Physical Examination, Vitals, Blood PressureFARS Assessment
- Urine analysis: F2-isoprostane
- Blood sample(s)
    - Analysis of (+)- EPI drug level and follistatin/myostatin levels
- BNP, Calcium, cardiac troponin I, CBC, ceruloplasmin, creatinine with eGFR, frataxin, glucose tolerance test, hemoglobin A1C, iron, mitochondrial complex I-V, potassium, PT/PTT, sodium, ST2, triglycerides, and liver panel (ALT, AST, and Alkaline Phosphatase)Neurology studies
    - Cerebellar MRI conducted on a non-FDA approved compact 3T MRI scanner and associated software and spinal cord MRI with volumetry, DTI, and Magnetic Resonance Spectroscopy (MRS) analysis
- Echocardiogram and electrocardiogram
- Cardiac MRI with BOLD/Perfusion analysis
- Urine pregnancy test for female subjects
- We may collect blood for off study evaluation if budget allows
    - Respiratory chain function, aconitase, catalase, PGC1α, SOD2 in blood

3.1 General Design

This is an open label, single center, and prospective phase II drug trial to assess the safety and efficacy of (+)-EPI in the treatment of Friedreich's ataxia. Duration of the trial including recruitment and final assessment is estimated at approximately 2 years: start date early 2016 to approximately late 2017, depending upon recruitment rate. Subjects will be evaluated at outpatient clinic visit appointments and interested qualified subjects will be consented and offered participation in this trial. Once consent has been obtained, baseline values will be established and subjects will begin treatment and follow-up over a 24 week period. Clinical and biochemical parameters shown in Table 1 will be assessed in each patient at baseline and at 3, 12 and 24 weeks after initiation of treatment. See Protocol Schedule of Events in Attachment II.

Subjects will be dispensed bottles of 25mg capsules of open-label (+) EPI with instructions for oral TID dosing to be taken on an outpatient basis. All subjects will start at 75 mg total daily dose. Those subjects showing improvement at 12 weeks as defined below, will continue at the starting dose level of 75 mg total daily dose (one, 25mg cap TID) to 24 weeks. Subjects showing no improvement or worsening at 12 weeks will have their dose escalated as described below for the remainder of the study, as tolerated.

Dose Escalation at 12 weeks: Those subjects showing no improvement or worsening in the 8 meter timed walk/FARS composite score or with no improvement in cardiac function by MRI at the 12-week clinical assessment, as compared to baseline, will have their dose increased at the 12 week visit to 150 mg total daily dose (two, 25mg caps TID) until 24 weeks, as tolerated.

Neurological Status Improvement Definition: If there is no change or the timed walk is longer at 12 weeks than at the baseline walk time or the FARs composite score has not changed or has

increased, as compared to baseline FARS composite score, this will be considered no improvement/worsening of neurological status. Improvement is defined as a greater than 1 point decrease in the FARS composite score, as compared to the baseline composite score [36].

Cardiac Status Improvement Definition: If the cardiac function is the same as baseline or worse at 12 weeks, this will be considered no improvement /worsening of cardiac LV structure/function.

Improvement in left ventricular structure and function by MRI is defined as any one of the following:

1. If LV mass was abnormally increased at baseline, reduction in LV mass of > 10% from baseline or
2. If LV ejection fraction was reduced at baseline, increase in LV ejection fraction of > 5% from baseline or
3. If LV end-diastolic volume was abnormally increased at baseline, reduction in LV end-diastolic volume of > 10% from baseline.

Neurologic Status: If spinal cord and cerebellar volumes (based on 3D volumetric MRI), fractional anisotropy (based on DTI), and metabolites (based on 3D MRS) are worse at 24 weeks compared to baseline, this will be considered no improvement /worsening of neurologic structure/function.

Worsening in neurologic structure and function by MRI is defined as any one of the following:

1. If spinal cord volumes were abnormally decreased at 24 weeks compared to baseline, reduction in cord volume of > 10% from baseline or
2. If cerebellar volumes were abnormally decreased at 24 weeks compared to baseline, reduction in cerebellar volume of > 10% from baseline or
3. If spinal cord fractional anisotropy was abnormally decreased at 24 weeks compared to baseline, reduction in fractional anisotropy of > 10% from baseline or
4. If cerebellar metabolite ratios (NAA/Cr, Cho/Cr, NAA/Cho) change > 5% from baseline at 24 weeks.

Study drug will be dispensed at the baseline visit for 12 (+2) weeks of dosing initially and resupplied at the 12 week visit for an additional 12 (+2) weeks of dosing at either the starting dose or escalated dose following re-assessment for changes in neurological and cardiac functions as described above.

Table 1

Neuromuscular	**Cardiac**	**Metabolic & Biochemical**
FARS (disability, ataxia, fine motor skills, peripheral neuropathy, and gait)	**MRI** (left ventricular mass, end-diastolic and end-systolic volumes, ejection fraction, strain analysis, myocardial fibrosis)	**Glucose tolerance test**
Brain MRI (spinal cord and cerebellar volumetry, DTI, and MRS)	**Blood Oxygenation Level Dependent (BOLD) MRI** (myocardial oxygenation)	**Whole Blood** Hemoglobin A1C Frataxin

		Heart Failure Biomarkers (BNP, and others) triglycerides
Magnetic Resonance Spectroscopy (lactate, N-acetylaspartate, and choline)	**Regadenoson-stress-perfusion MRI** (myocardial perfusion index)	**Leukocytes** ETC Complexes Other relevant proteins Bioenergetics
	M-mode, 2D, 3D, tissue Doppler (left ventricular mass, volumes, systolic and diastolic function)	**Urine** F2-isoprostane
	Echocardiography (strain imaging)	

3.2 Primary Study Endpoints

Neurological:

- Change from baseline score using Friedreich's Ataxia Rating Scale (FARS Assessment) tool at 12 and 24 weeks.
- Changes in spinal cord fractional anisotropy, based on axial DTI measurements prescribed at the C1, T1, and L1 levels at 24 weeks.

Cardiac:

- Changes in Cardiac MRI from baseline to 12 and 24 weeks:
    - Left ventricular structural and functional characteristics (systolic function by ejection fraction and systolic strain, diastolic function, LV mass to determine ventricular hypertrophy and degree of heart failure, myocardial fibrosis by late gadolinium enhancement).
- Changes in Echocardiogram from baseline to 24 weeks:
    - Left ventricular mass, volumes, systolic and diastolic function, and strain.
- Changes in Electrocardiogram from baseline to 24 weeks to assess for electrophysiological improvement of the myocardium.
- Changes in biomarkers for heart failure and hypertrophy (ST2, BNP, Cardiac Troponin) from baseline, at 12 weeks and 24 weeks.

3.3 Secondary Study Endpoints

- Changes in spinal cord and cerebellum volume by MRI from baseline to 24 weeks after initiation of therapy:
    - Spinal cord and cerebellar volumes based on high-resolution 3D volumetric T1- and T2-weighted datasets.

- Metabolic change from baseline at 12 weeks and 24 weeks measured by:
    - Glucose tolerance test, fasting blood glucose and hemoglobin A1C at baseline, 12 (+/-), and 24 (+/-) weeks to assess for improvement of diabetes mellitus.
- Change in mitochondrial biomarkers from baseline to 24 weeks:
    - Assess for upregulation of relevant mitochondrial proteins (frataxin and ETC complexes I-IV) and (ii) reduction of urine F2-isoprostane as a measure of oxidative stress.

3.4 Exploratory Endpoints

- Change in cardiac stress-regadenoson and Blood Oxygenation Level Dependent (BOLD) MRI from baseline at 12 and 24 weeks after treatment:
    - Myocardial oxygenation by BOLD sequence on 3T MRI
    - Myocardial perfusion index via cardiac stress-regadenoson MRI from baseline to 24 weeks
- Change in cerebellar metabolites (Cho, Cr, NAA), based on 3D MRS data obtained over the posterior fossa and upper cervical cord.
- Change in follistatin and myostatin levels from baseline at 3 (+/-1), and 24 (+/-1) weeks as a measure of muscle improvement.

3.5 Primary Safety Endpoints

Safety endpoints will include:

- Distribute a diary to each study participant in order to date and record symptoms of adverse events along with drug intake. All participants will be asked to contact study coordinator or PI via phone in the case of an adverse event, which will then be documented in the source documents. Participants will be asked to bring the diary along with their drug bottles to each clinic visit.
- Comparison between treatment groups of the incidence and types of reported adverse events including increase in migraine headaches, bruising or bleeding.

  The consumption of cocoa products containing the epicatechins has been reported to be associated with increased likelihood of migraine development [37]. However, the migraine effect has not been validated in subsequent clinical trials, and, if it exists, has been attributed to the presence of phenylethylamine [38].

  Anti-clotting like effects have been described for foods containing members of the flavonoid family including epicatechin, such as cocoa, tea, and guarana. There is no report as to the effects that epicatechin per se may have on phenomena such as platelet aggregation or clotting times [14, 38, 39].

  The modest effects on platelet aggregation of food containing polyphenols has been cited as one basis for beneficial crdiovascular effects. To our knowledge, no adverse

event involving platelet inhibition has been described in any study of foods or their extracts of cocoa,guarana, or tea. Nonetheless, we deemed it prudent to exclude patients with thrombocytopenia or clotting disorders.

- Potential risk based on the biological activities of (+)-epicatechin includes hypotension through presumed mechanism of nitric oxide (NO) mediated vasodilation [40]. Of note is the fact that so far, no blood pressure reducing effects by (-)-epicatechin have been reported in normotensive subjects. With cocoa based studies, blood pressure reducing effects are only reported in humans that have high blood pressure [41]. In the study by Ottaviani et al., the vasodilating effects of (+)-epicatechin in humans were only 25% of those of (-)-epicatechin when given at the same doses (1.5 mg/kg) [21]. There is the possibility that patients undergoing pharmacologic treatment for high blood pressure if given epicatechin may develop hypotension through additive or synergistic effects.
- Guarana effects on toxic and behavioral parameters were assessed in rats and mice subsequent to acute and chronic administrations. Experimental parameters included tests for antioxidant capacity in vitro, in vivo, toxicological screening, body weight, motor activity, death rate, and histopathological examination of viscera. Guarana demonstrated antioxidant effects even at low concentrations by inhibiting lipid peroxidation. At high doses (1-2 g/kg IP and PO) it did not induce significant alterations in toxicological parameters. Consumption of liquids containing guarana evidenced unaltered weight of the animals even after prolonged (23 months) administration. The percentage mortality was equivalent in control and guarana groups. The absence of toxicity of guarana was also demonstrated by histopathological examination, with no alterations being detected in heart, lungs, stomach, small and large intestine, liver, pancreas, kidneys, bladder and spleen [42].
- A 10 day study in which rats were dosed at 100 mg/kg/day, approximately 50X our proposed dose in humans, demonstrated that (+)-epicatechin at this high dose demonstrated no adverse effects with respect to appetite, behavior, alertness , or appearance. No abnormalities of any organs were observed at necropsy [43].
- (+)-Epicatechin. Campos et al recently evaluated the effects of standardized Guarana encapsulated dried extract on fatigue, sleep quality, anxiety, depression symptoms, and menopause in a group of breast cancer chemotherapy patients. Patients with progressive fatigue after their first cycle of chemotherapy were randomized to receive either guarana 50 mg orally twice daily (n=32) or placebo (n=43) for 21 days. After a 7-day washout period, patients were crossed over to the opposite experimental arm. All patients were evaluated on days 1, 21, and 49. Guarana significantly improved fatigue measures vs. placebo on days 21 and 49. Guarana treatment did not produce any Common Terminology Criteria for Adverse Events grades 2, 3, or 4 toxicities and did not worsen sleep quality or cause anxiety or depression [44].

Overdose

- Neither the effects of overdose of (+)-epicatechin nor an antidote to overdose are known. For reference, the intraperitoneal (-)-epicatechin median lethal dose (LD_{50}) reported for mice is 1000 mg/kg as stated in the Material Safety Data Sheet provided by suppliers such as Sigma-Aldrich.

Pregnancy and Lactation

There are no known adverse effects of natural products containing epicatechin in pregnant women. In a developmental toxicity study in pregnant rats using a green tea extract, the no-observable-adverse-effect level (NOAEL) corresponded to 100 mg (-)-epicatechin/kg (the highest dose tested). No information is available on levels of (-)-epicatechin in breast milk.

Pregnancy testing will be done at baseline and repeated at the 12th week clinic visit. Patients who have a positive pregnancy test, are pregnant or breastfeeding will be excluded from the study.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

Subjects must fulfill all of the following criteria:

Inclusion criteria:

- Confirmed diagnosis of Friedreich's ataxia by *FXN* genetic testing and/or Frataxin enzyme analysis
- Between age 10 and 50 years of age, inclusive
- Body weight of 25 kg or higher
- Minimum of one affected organ (cardiac or neurological) system, as evidenced by clinical signs/symptoms
- Disease duration ≤7 years, based on onset date of FA symptoms
- Has no known contraindication to gadolinium contrast such as severe allergy or GFR <30 ml/min/m2.
- Has no known contraindication to non-contrast MRI evaluation such as pacemaker or magnetically active metal fragments.
- Women of childbearing age must:
    - Have a negative pregnancy human chorionic gonadotropin test prior to receiving study drug.
    - Agree to use contraception for the duration of the study drug dosing, plus 1 month after completion of the study.

4.2 Exclusion Criteria

Exclusion criteria:

- Advanced cardiac failure, NYHA Class IV.

- Clinically significant comorbidities that may also lead to cardiomyopathy, for example long standing hypertension, familial cardiomyopathy.
- Clinically significant comorbidities that would, in the opinion of the investigators, compromise the interpretation of test results.
- Pregnant, breast-feeding or planning to become pregnant during study timeframe.
- Patients with contraindications to regadenoson, i. e. second- or third-degree atrioventricular (AV) block or sinus node dysfunction. Has received an investigational drug within thirty (30) days of baseline visit.
- Thrombocytopenia (<125 x 10^9/L) or prolonged PT/PTT at baseline.
- Clinically significant hypotension (systolic BP<90) due to heart failure or other conditions.

4.3 Subject Recruitment, Enrollment and Screening

Subjects may be recruited:

- From the Principal Investigator or Sub-Investigator's clinical practices; may also be identified by Study Coordinator.
- By physician referrals within Mayo Clinic, by referrals from non-Mayo Clinic physicians, by contact made via the Mayo Clinical Trials database with a link to the national ataxia foundation web site, or by patient inquiries via contacts listed on the www.clinicaltrials.gov registry.
- Mayo Clinic investigators using social media (e.g. Twitter, Facebook, YouTube, etc.) as a recruitment method must have the research reviewed and approved by the Mayo Clinic Public Affairs/Center for Social Media.
- New projects proposing the use of social media as a recruitment method will contain a statement from the PI in the IRBe application or the research project proposal that the use of social media for recruitment of research subjects has been reviewed and approved by the Mayo Clinic Public Affairs/Center for Social Media.
- Investigators at institutions for which the Mayo Clinic IRB is the IRB of record will include within the IRB application documentation of review and approval by the relying organization for the use of social media, or documentation that such review and approval is not required by the institution.

Enrollment

Study participants with genetically confirmed diagnosis of FA who meet inclusion criteria and do not meet exclusion criteria will be enrolled according to the most current IRB approved version of this protocol. Adequate time will be allowed for review of consent/assent and to ask questions about the study prior to enrollment. Subjects aged 13-17 will also sign an IRB approved assent. A copy of the signed consent/assent form will be provided to the subject/LAR and the original will be retained in the Investigator's study file. A copy of the signed forms will also be scanned into the subject's Mayo Clinic electronic medical record.

Documentation of informed consent/HIPAA authorization will involve the use of the Research Participant Tracking (PTrax) Digital Signature Capture technology for research informed consent forms/HIPAA authorization forms. This is an institutionally approved process for obtaining

consent/HIPAA authorization only while the subject, and/or the subject's representative, is in the physical presence of the person authorized to obtain consent. The study team may print a copy of the signed consent form/HIPAA authorization form for the subject or their representative. The consent form/HIPAA authorization form will also be available to the subject via the patient portal. **Note:** If the subject or their representative prefers not to use the Digital Signature Capture technology, the study team will provide a paper consent form/HIPAA authorization form for signature.

4.4 Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subject Withdrawal Criteria

- The end of the trial for a patient will be defined for this study as (1) completion of the final 24 week evaluation, (2) death, (3) withdrawal of consent, (4) if the patient is lost to follow-up at 12 weeks clinic visit, the date of the patient's last visit for which data is available.
- Patients may be withdrawn from this trial for the reasons provided below:

Withdrawal for Adverse Event

- A patient who experiences an adverse event during the study drug administration that the Investigator judges to be related to the study drug and poses a significant risk to the patient will have the study drug discontinued. All scheduled data will be collected and the patient will be included in all safety analyses.

Withdrawal of Consent

- Patients for whom Informed Consent is withdrawn will have study drug discontinued (if the decision occurs during the study period). No further data collection will be done without written consent for follow-up data collection. All available data on these patients will be included in the safety analyses.
- Investigator Decision to Withdraw Patient Should the Investigator determine that it is in the best interest of a patient to be withdrawn from the trial, the drug will be immediately discontinued (if the decision occurs during the study period). All scheduled data deemed appropriate by the Investigator will be collected and the patient will be included in all safety analyses.
- Pregnancy; If a subject's pregnancy test is positive at 12 weeks, subject will be discontinued from further study participation and any dispensed study medication will be retrieved from the subject. Subject and child will be followed for potential adverse effects of study drug dosing.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Even though a subject has withdrawn from the study, it may be important to collect some follow-up or survival data on such subjects throughout the protocol defined follow-up period. Such data is

important to the integrity of the final study analysis since early withdrawal could be related to the safety profile of the study drug. If a subject withdraws consent to participate in the study, for subject safety reasons, attempts should be made to obtain permission to collect follow up information whenever possible.

4.5 Replacement of Discontinued or Withdrawn Subjects

Subjects who discontinue or are withdrawn prior to completing twenty-four (24) weeks of treatment will be replaced, to achieve a target of ten subjects completing 24 weeks of dosing with study medication. The number replaced may be limited to the available inventory of (+) Epicatechin capsules.

5 Study Drug

5.1 Description

(+) EPI will be manufactured by Syngene Corporation in Bangalore, India for Cardero Therapeutics and provided without charge to study subjects. The drug product will be provided to Mayo Clinic as 25mg gelatin capsules, packaged in 42 capsules per high density polyethylene bottle.

5.2 Treatment Regimen

The starting total daily dose will be 75 mg per day (one 25mg capsule taken TID) Dose escalation for some subjects, as described above, will occur at 12 weeks to 150mg total daily dose (two, 25mg capsules taken TID).

Starting Dose	**Dispense 12 week supply + 2 weeks overage @ BL visit**	**Increase Dose at 3 months (for some subjects)**	**Dispense 12 week supply + 2 weeks overage at 12 week Clinic Visit**	**Total # Bottles (& caps) Dispensed to each Subject**
75 mg/day one cap TID 3 caps/day	7 bottles of 42 (294 caps)	150 mg/day two caps TID 6 caps per day	7 bottles of 42 caps (294 caps) OR escalated dose: 14 bottles of 42 caps (588 caps)	14 bottles (588 caps) --- 21 bottles (882 caps) Escalated dose

5.3 Preparation and Administration of Study Drug

- The study drug will be shipped to, stored by and dispensed from the Mayo Clinic Out-Patient Research Pharmacy located in the [redacted]
- The study drug will be dispensed to subject/LAR, labeled to be taken orally as described in section 5.2 above.
- Certificates of Analysis and clinical supply expiry date extensions as provided by Cardero Therapeutics, based on ongoing, concurrent stability studies, should be emailed to [redacted]

5.4 Subject Compliance Monitoring

Study medication will be dispensed as part of the Baseline and 3 month study visits at Mayo Clinic, to be taken home with the subject. A dosing and adverse event diary will be provided to the subjects, who will be asked to record the dates/times of medication intake and any unusual symptoms. The subjects will be asked to bring their diary and the previously dispensed bottles (empty, partial and unused) to the 3 month visit for review by study staff. Parents or guardians of participants younger than 18 years of age will be asked to supervise and/or provide the medication to their child and ensure the diaries are completed.

In addition to the dosing diary, the subjects/LARs will be contacted periodically between on-site visits to assess doses are being taken as prescribed.

A count of capsules returned at the 3 month visit will be performed by a study team member/pharmacy, and the number of returned capsules and compliance will be documented.

The blood levels of (+)-EPI will be measured subsequent to the visits to provide the most accurate measure of dosing compliance.

If it is determined by study staff by review of diary, returned amounts and interview that the subject did not take 50% or greater of the daily dose for 3 days in any given week, the subject will be asked to become compliant. If it is determined by the above criteria to be non-compliant a second time, the subject will be dropped from the study.

Completed dosing diaries will be retained with the site's study source documents.

5.5 *Prior and Concomitant Therapy*

All concomitant medications will be allowed and at the time of enrollment and through study period will be recorded and current medications will be recorded at each clinical follow-up.

5.6 Packaging

25 mg capsules of open-label study medication will be packaged 42 capsules per bottle. The bottles will be subsequently labeled by the Research Pharmacy for each subject, based on a written prescription by a Study Investigator. A 3 month supply will be dispensed at the Baseline visit and again at the 3 month visit, as specified in Section 3.1. An overage amount (2 week supply) will be dispensed to allow for flexibility in visit scheduling.

In addition to dosing directions, the bottles shall bear a label with the statement *"Caution: New Drug--Limited by Federal (or United States) law to investigational use."*

5.7 Masking/Blinding of Study

Not applicable; the study medication will be open label.

5.8 Receiving, Storage, Dispensing and Return of Study Medication

5.8.1 Receipt of Drug Supplies

The Mayo Clinic Research Pharmacy will maintain drug shipment and dispensing accountability records, including shipping receipts/packing slips for incoming shipments of (+) EPI and dates/number of capsules/bottles and the lot numbers dispensed to subjects.

Expiry dating/retest dates will be provided by Cardero Therapeutics, based on ongoing stability testing. Documentation of expiry/re-test dates will be retained with study records.

Subject dosing/AE diaries will be provided to the subject/LAR by the study team with completion instructions. Subjects will be asked to bring their completed diaries back to the site, with any full or partial bottles to facilitate reconciliation.

The quantity of capsules dispensed, taken, returned or discarded by the subject will be reconciled by the study team during visits to determine compliance with the prescribed dosing regimen.

Study drug reconciliation will be documented in study records.

5.8.2 Storage Conditions

Capsules will be stored in HDPE bottles as packaged by Syngene, and maintained at 2 to 8 degree storage within the Mayo Clinic Research Pharmacy. Subjects/LAR will be advised to keep bottles refrigerated in their homes.

5.8.3 Dispensing of Study Drug

A prescription will be written, signed by an Investigator and faxed to the Research Pharmacy for the baseline and 3 month visit dispensing. The subject/LAR will either pick up the medication at the Out-Patient Pharmacy or if picked up in advance by a study team member, will be provided to the subject by the study team with a dosing/AE diary.

5.8.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug amounts shipped, dispensed, returned, and remaining. This reconciliation will be included in study files. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Any investigational drug destroyed on site according to institutional waste policy will be documented by the Research Pharmacy.

6 Study Procedures

The schedule of events for patients enrolled in this trial is shown in Attachment II. See Section 3 for visit descriptions.

7 Statistical Plan

Statistical analysis will be performed using SAS version 9.3 (SAS Inc., Cary, NC). All tests will be two sided and p-values less than 0.05 will be considered statistically significant.

7.1 Sample Size Determination

A recent single-center open-label pilot study enrolled eight adults with genetically-confirmed Becker muscular dystrophy, who received 100 mg/day of EPI orally for 8 weeks. The data showed linear decrease in plasma B-Type Natriuretic Peptide (BNP) levels, a validated biomarker of congestive heart failure [15]. Assuming a common standard deviation of 234.3 as seen in the Becker pilot study, with a sample size of 10 patients we should have 80% power to detect an effect size (on BNP and possibly other biomarkers) of 1at a 5% level of significance based on a paired t-test. NQuery Advisor version 6.0 was used for sample size calculations.

7.2 Statistical Methods

Descriptive Statistics
All numerical data will be imported into a dedicated application of highly-innovative multivariate pattern recognition software developed at Mayo by [redacted]) that provides an integrated analysis of outcome measures after statistical adjustment for one or more relevant covariates. Descriptive summaries will be reported as frequencies and percentages for categorical variables and as mean (standard deviation) or median (inter-quartile range) for continuous variables of interest.

Handling of Missing Data
Data edit checks will be performed to assess outliers or any inconsistencies done at the time of data entry. No imputations will be performed for any missing data.

Multiplicity
Given the smaller sample size, no formal adjustment for multiple comparisons will be performed. We will however, report both raw (unadjusted) and false discovery rate corrected p-values.

Primary Hypothesis:
In light of the common underlying pathogenic mechanism of energy deficit and oxidative damage, we hypothesize that the induction of mitochondrial biogenesis and bioenergetics along with simultaneous upregulation of the cellular antioxidant defenses is a strategy capable to ameliorate MtD. To test this hypothesis we propose to conduct a phase II drug clinical trial to test the efficacy of a novel synthetically-produced form of Epicatechin (EPI) in patients with Friedreich's Ataxia (FA). For continuous endpoints such as FARS assessment, we will calculate changes from baseline to 12 weeks and 24 weeks. These will be summarized and mean (standard deviation) or median (inter-quartile range). We will also report 95% confidence intervals. Formal comparison to assess

efficacy of intervention will be performed using paired t-test or Wilcoxon signed rank test as appropriate. Analysis will also be performed using percent changes from baseline to 12 week and 24 week to account for baseline discrepancies. Data will be displayed graphically using scatter plots to show the trends over time. Categorical outcomes will be compared using McNemar's test as a test of paired proportions to account for paired nature of the data.

Secondary Hypothesis 1:

- Upregulate mitochondrial biogenesis via activation of the transcription factor PGC-1α [9] which is downregulated in FA [45]; this may lead to higher levels of frataxin as well as higher levels of Fe-S proteins critical for ATP production.
- Upregulate endogenous antioxidant mechanisms (SOD2, catalase) controlled by the Nrf2 pathway [18] which is inhibited in FA cells [46]; this may enhance FA cells resistance to iron-catalyzed oxidative damage.
- Promote fatty acid oxidation through regulation of SIRT3 signaling [9, 19] which is inhibited in FA cardiac cells and believed to contribute to the FA cardiomyopathy [47].
- Enhance insulin sensitivity through the PI3K/AKT pathway and AMPK [10] which may further improve metabolic dysfunction in FA.
- Through the basic mechanisms described above, (+)-EPI treatment will ultimately prevent further loss of neuronal, cardiac, and pancreatic β cells and thereby delay or arrest FA neurodegeneration, cardiomyopathy and diabetes.

Statistical analysis approach for secondary endpoint will be similar to approach listed above for primary endpoints.

Interim Analysis

Interim analysis will be performed after 50% i.e. 5 out of 10 patients have completed 12 week follow-up. Sample size at interim analysis is too small to reach required p-value for stopping decision based on O'Brien Flemming Stopping boundary criterion (i.e. p-value less than 0.0054 for stopping at interim analysis. Thus decision of stopping versus continuation of the study will be based on clinical significance rather than statistical significance. We will consider treatment to be efficacious if the cardiac MRI, neurologic MRI, and /or FARS score improve by greater than 30% at 3 months.

7.3 Subject Population(s) for Analysis

Protocol-compliant population: Any subject who participated and received the protocol required study drug exposure.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others

(including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, AND

- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event

An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject. Adverse events are classified as serious or non-serious.

Serious Adverse Event

An adverse event 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
- Is considered a life-threatening adverse event
- 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 patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as three (3) days following the last administration of study treatment, based on 2 hour half-life of EPI.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A laboratory abnormality should be documented as an adverse event if the laboratory value remains outside of the normal range on repeat testing and the subject's personal physician or PI, believe might reasonably be related to study medication. If the subject's personal physician or PI considers the laboratory abnormality unsafe/clinically significant, the study treatment might be discontinued. If the laboratory abnormality is considered treatable, it will be treated appropriately. The laboratory abnormality will be followed until it normalizes or no longer is considered unsafe and needing a follow up.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

8.2 Recording of Adverse Events

During each contact with the subject, the study team will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded in the source document, and also in the appropriate adverse event section of the case report form (CRF) or in a separate adverse event worksheet. All changes in symptoms, and diagnostic, laboratory or procedure results that potentially are considered related to study medication should recorded in the source documents. The subject's AE diary will be considered a study source document and retained by the site within the subject's study file for monitoring and inspectional purposes.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still

ongoing at the end of the study period must be followed, to determine the final outcome. Any serious adverse event that occurs after the study period and considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and/or log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

The sponsor-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report all unexpected, serious suspected adverse reactions to the FDA according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Stopping Rules

The study will be halted if Serious Adverse Events which are felt by the PI to possibly be related to treatment with study medication occurs in 2 or more patients. Any adverse event with the outcome of death that is felt caused by or possibly related to treatment with study medication will cause the study to be stopped.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, subject diaries, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Database

Data Management
A RedCap database will be used to collect the data intended for analysis and entries will be available for review by the ORRS study monitor as well as for regulatory inspectors.

Data Security and Confidentiality
The RedCap study database will be maintained on password protected computers, hosted on Mayo Clinic servers, which are backed up on a daily basis according to Institutional Policies.

Data Quality Assurance
Data will be extracted from source documents and entered into the study database. The Study Coordinator or study team member so delegated by the PI will assure the accuracy and integrity of the data and the PI will evaluate the data and the supporting source documents periodically.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and study regulatory documents.
The sponsor-investigator will retain the specified records and reports for the longest of these retention periods:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted]

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

As a service to the sponsor-investigator and to assist with the sponsor regulatory obligation to monitor study progress, this study will be periodically monitored during the conduct of the trial by staff from the Mayo Clinic Office of Research Regulatory Support. Clinical trial monitoring may include but will not necessarily be limited to review of the study regulatory documents, source data and database entries throughout the duration of the study to help ensure the validity and integrity of the data. Original informed consent forms will be reviewed. Written monitoring reports with findings and recommended and suggested corrective actions will be provided to the sponsor.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring by ORRS and audits by the IRB Compliance Unit, if indicated, as well as inspections by authorized representative of government regulatory agencies such as FDA of all study related documents (e.g. source documents, regulatory documents, data collection instruments, drug accountability records and study data etc.). The investigator will

ensure access is granted for inspection of applicable study-related facilities (e.g. pharmacy, diagnostic laboratories, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to applicable United States government regulations and Institutional research policies and procedures.

This protocol and any amendments as well as subject materials such as the informed consent/assent forms will be submitted to a properly constituted Mayo Clinic Institutional Review Board (IRB for review and formal approval. The decision(s) of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent (or assent) form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The formal consent of a subject, using the IRB approved consent or assent form, must be obtained before subject undergoes any study procedure. The consent form must be signed and dated by the subject or the subject's legally authorized representative, as well as the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study will be financed through a grant from the Mayo Clinic Children's Research Center Pediatric Team Science Award. Study medication will be provided by Cardero Therapeutics. Costs associated with the study-specific laboratory tests performed by UC-San Diego will be paid for by Cardero Therapeutics.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

13 Publication Plan

This study will be registered on the ClinicalTrials.gov registry prior to the first subject being enrolled. Study results will subsequently be posted to the registry within 12 months of final data collection for the primary outcome measure.

Study results will be analyzed and manuscripts prepared for submission to the appropriate peer-reviewed scientific journals. The NCT number linking to the clinicaltrials.gov record will be included in manuscripts.

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15 Attachments

15.1 Attachment I Friedreich's Ataxia Rating Scale (FARS)

15.2 Attachment II Protocol Schedule of Events

Attachment I
Friedreich's Ataxia Rating Scale

NINDS CDE Notice of Copyright
Friedreich Ataxia Rating Scale (FARS)

Availability:	**The instrument is freely available here: http://www.ataxia-study-group.net/html/about/ataxiascales/fars**
Classification:	Core
Short Description of Instrument:	The Friedreich Ataxia Rating Scale (FARS) is made up of a measure of ataxia, an activities of daily living subscale and a neurological subscale. This scale also includes the 8m walk at maximum speed (8MW), the 9-hole peg test (9HPT), and low-contrast letter acuity. **Strengths:** FARS scores correlate significantly with functional disability, activities of daily living scores and disease duration. FARS has a large effect size and requires fewer patients for an equivalently powered study. **Weaknesses:** This scale captures a particular dimension of neurologic function in FA, so as a composite measure, it is not good enough to predict disease status.
Scoring:	The scores from the three subscales are added to generate a total score ranging from 0 to 159, with a higher score indicating a greater level of disability. Functional Staging for Ataxia subscale scoring: 0 = Normal - 6.0 = Confined to wheelchair or bed with total dependency for all ADL. Total disability. ADL subscale scoring: 9 items scored from 0 to 4. Neurological Examination subscale scoring: 5 items scored from 0 to 2 or 0 to 5, depending on the specific parameter.
References:	**Key Reference:** Subramony SH, May W, Lynch D, et al. Measuring Friedreich ataxia: interrater reliability of a neurologic rating scale. Neurology. 2005;64:1261–2. **Other References:** Delatycki MB. Evaluating the progression of Friedreich ataxia and its treatment. J Neurol. 2009. 256(Suppl 1):36-41. Fahey MC, Corben L, Collins V, Churchyard AJ, Delatycki MB. How is disease progress in Friedreich's ataxia best measured? A study of four rating scales. J Neurol Neurosurg Psychiatr. 2007 Apr; 78(4):4113-413. Lynch DR, Farmer JM, Tsou AY, Perlman S, Subramony SH, Gomez CM, Ashizawa T, Wilmot GR, Wilson RB, Balcer LJ. Measuring Friedreich ataxia: complementary features of examination and performance measures. Neurology. 2006 Jun 13;66(11):1711-6. Subramony SH, May W, Lynch D, Gomez C, Fischbeck K et al. Measuring

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Short Description of Instrument:	
Scoring:	
References:	
	The Nine Hole Pegboard Test
Short Description of Instrument:	**Construct measured:** Upper extremity function **Generic vs. disease specific**: Generic **Means of administration:** In person by a trained examiner **Intended respondent:** Patient **# of items:** N/A **# of subscales and names of sub-scales:** N/A **# of items per sub-scale:** N/A

	Scoring: Both the dominant and non-dominant hands are tested twice within a single testing session. The total time to complete the task is recorded. The score for the 9-HPT is an average of the four trials. The two trials for each hand are averaged, converted to the reciprocals of the mean times for each hand and then the two reciprocals are averaged. This score can be used individually or used as part of the MSFC composite score. **Background:** The 9-HPT is a brief, standardized, quantitative test of upper extremity function. It is the second component of the MSFC to be administered at each visit. Both the dominant and non-dominant hands are tested twice. Two consecutive trials with the dominant hand are immediately followed by two consecutive trials with the non-dominant hand.
Psychometric Properties:	**Strengths/Weaknesses:** There has been increasing recognition of the usefulness of measuring arm and hand function in clinical studies. The 9-HPT is one of the most frequently used measures of upper extremity function in MS. It is responsive to changes at the upper level of performance but not when impairment is severe. **Psychometric Properties:** The 9-HPT has high inter-rater reliability and good test-retest reliability. There is evidence for concurrent and convergent validity as well as sensitivity to detect minor impairments of hand function. Performance on the 9-Hole Peg Test may be sensitive to practice effects, that is, patients often display poorer performance when first tested due to lack of familiarity with the task. It is recommended that three or four administrations be given prior to a baseline assessment if accurate (rather than comparative) assessments of change over time are needed. **Administration:** The 9HPT is administered in person by a trained examiner, who need not be a physician or nurse. Administration time varies depending upon the ability of the patient, but typically total administration time is approximately 10 minutes or less.
	Low Contrast Letter Acuity
Short Description of Instrument:	**Construct measured:** Low contrast letter acuity **Generic vs. disease specific**: Generic **Means of administration:** Assessment **Intended respondent:** Patient **# of items:** N/A **# of subscales and names of sub-scales:** N/A **# of items per sub-scale:** N/A
Scoring:	**Scoring:** Charts are scored based on the number of letters identified correctly. This format provides continuous scoring and may allow Sloan charts to capture losses of contrast at small letter sizes. **Background:** Low contrast acuity testing provides information on patient- reported aspects of vision. This testing is performed using low-contrast Sloan letter charts. Each chart corresponds to a different contrast level (shade of gray letters on white/retroilluminated background).

References:	**Key Reference:** Balcer LJ, et al. Contrast letter acuity as a visual component for the Multiple Sclerosis Functional Composite. Neurol. 2003; 61:1367-73. **Other References:** Balcer LJ, et al. New low-contrast vision charts: reliability and test characteristics in patients with multiple sclerosis. Mult Scler June 2000; 6(3):163-171.
Psychometric Properties:	**Strengths/ Weaknesses:** Sloan charts have a standardized format based on Early Treatment Diabetic Retinopathy Study visual acuity charts, which are the standard used for ophthalmology clinical trials. **Psychometric Properties:** Inter-rater agreement was described with the intraclass correlation coefficient (ICC) and comparison of mean scores. Excellent inter-rater agreement (ICC=0.86-0.95) was demonstrated at each contrast level among MS patients (n=100) and visually-asymptomatic volunteers (n=33). Average letter scores at the lowest contrast level (0.6%) were highly variable in the MS group, even among patients with visual acuities of 20/20 or better, and among those who required no assistance for ambulation.

SUPPLEMENTAL DATA: E - APPENDIX I (E)A-1): RATING SCALE FOR FRIEDREICH'S ATAXIA

I. FUNCTIONAL STAGING FOR ATAXIA

Increment by 0.5 may be used if the status is about the middle between two stages.

STAGE ☐☐

STAGE 0: Normal.

STAGE 1.0: Minimal signs detected by physician during screening. Can run or jump without loss of balance. No disability.

STAGE 2.0: Symptoms present, recognized by patient, but still mild. Cannot run or jump without losing balance. The patient is physically capable of leading an independent life, but daily activities may be somewhat restricted. Minimal disability.

STAGE 3.0: Symptoms are overt and significant. Requires regular or periodic holding onto wall/furniture or use of a cane for stability and walking. Mild disability. (Note: many patients postpone obtaining a cane by avoiding open spaces and walking with the aid of walls/ people etc. These patients are grades as stage 3.0)

STAGE 4.0: Walking requires a walker, Canadian crutches or two canes. Or other aids such as walking dogs. Can perform several activities of daily living. Moderate disability.

STAGE 5.0: Confined but can navigate a wheelchair. Can perform some activities of daily living that do not require standing or walking. Severe disability.

STAGE 6.0: Confined to wheelchair or bed with total dependency for all activities of daily living. Total disability.

II. 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: ☐

III. NEUROLOGICAL EXAMINATION (rate each item on the basis of the patient status during examination. To the extent possible, sequential patient examinations should be carried out at the same time of the day. If the patient is taking any medication, the examination should be carried out prior to dosing, or at a fixed time following the dosing based on the maximum expected therapeutic response. Increments of 0.5 may be used if examiner feels an item falls between 2 defined severities)

A. BULBAR

1. Facial Atrophy, Fasciculation, Action Myoclonus, and Weakness: ☐

0 - None
1 - Fasciculations or action myoclonus, but no atrophy.
2 - Atrophy present but not profound or complete.
3 - Profound atrophy and weakness.

2. Tongue Atrophy, Fasciculation, Action Myoclonus and Weakness: ☐

0 - None.
1 - Fasciculations or action myoclonus, but no atrophy.
2 - Atrophy present but not profound or complete.
3 - Profound atrophy and weakness.

3. Cough: (Patient asked to cough forcefully 3 times) ☐

0 - Normal.
1 - Depressed.
2 - Totally or nearly absent.

4. Spontaneous Speech (ask the patient to read or repeat the sentences "The President lives in the White House" or "The traffic is heavy today": ☐

0 - Normal.
1 - Mild (all or most words understandable).
2 - Moderate (most words not understandable).
3 - Severe (no or almost no useful speech).

TOTAL BULBAR SCORE: ☐

B. UPPER LIMB COORDINATION

1. Finger to Finger Test (The 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):

Right ☐ Left ☐

0 - Normal.
1 - Mild oscillations of finger (< 2 cm.).
2 - Moderate oscillations of finger (2-6 cm.).
3 - Severe oscillations of finger (> 6 cm.).

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 > 3 sec.):

Right Left

0 - None
1 - Mild (< 2 cm. amplitude).
2 - Moderate (2-4 cm. amplitude or persisting through movement).
3 - Severe (> 6 cm. & persisting through movement).
4 - Too poorly coordinated to perform task.

3. Dysmetria (Fast Nose-Finger) Test: (Assess dysmetria: The patient touches tip of examiner's finger 8 times as rapidly as possible while the examiner moves his finger and stops at different locations at about 90% reach of the patient. Assess dysmetria – i.e. inaccuracy of reaching the target- at examiner's finger):

Right Left

0 - None.
1 - Mild (misses 2 or fewer times).
2 - Moderate (misses 3-5 times).
3 - Severe (misses 6-8 times.).
4 - Too poorly coordinated to perform task.

4. Rapid Alternating Movements of Hands (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 > 7 sec. add 1 to score. Use stopwatch):

Right Left

0 - Normal.
1 - Mild (slightly irregular or slowed).
2 - Moderate (irregular and slowed).
3 – Too poorly coordinated to perform task.

5. Finger Taps (index fingertip-to-thumb crease; 15 reps as fast as possible; practice 15 reps once before rating; if time > 6 sec., add 1 to rating. Use stopwatch):

Right Left

0 - Normal.
1 - Mild (misses 1-3 times).
2 - Moderate (misses 4-9 times).
3 - Severe (misses 10-15 times).
4 - Cannot perform the task.

TOTAL UPPER LIMB COORDINATION SCORE

C. LOWER LIMB COORDINATION

1. Heel Along Shin Slide (under visual control, slide heel on the contralateral tibia from the patella to the ankle up and down, 3 cycles at moderate speed, 2 sec./cycle, one at a time. May be seated with contralateral leg extended or supine but perform same way each time. Circle which: supine seated):

Right Left

0 - Normal (stay on shin).
1 - Mild (abnormally slow, tremulous but contact maintained).
2 - Moderate (goes off shin a total of 3 or fewer times during 3 cycles).
3 - Severe (goes off shin 4 or more times during 3 cycles).
4 - Too poorly coordinated to attempt the task.

2. Heel-to-Shin Tap (patient taps heel on midpoint of contralateral shin 8 times on each side from about 6-10", one at a time. May be seated with contralateral leg extended or supine but perform the same way each time. Circle which: supine seated):

0 - Normal (stays on target).
1 - Mild (misses shin 2 or < times).
2 - Moderate (misses shin 3-5 times).
3 - Severe (misses shin > 4 times).
4 - Too poorly coordinated to perform task.

Right ☐ Left ☐

TOTAL LOWER LIMB COORDINATION SCORE ☐

D. PERIPHERAL NERVOUS SYSTEM

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

0 - None.
1 - Present - mild/moderate
2 - Severe/total wasting

Right ☐ 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).

Right ☐ Left ☐

3. Vibratory Sense (Educate patient regarding the sensation. Tested with 128 cps tuning fork set to near full vibration; eyes closed; test over index finger and great toe. Abnormal < 15 seconds for toes and <25 seconds for hands):

	Right	Left
Time felt for toes:	_____	_____
Time felt for fingers:	_____	_____

0 - Normal.
1 - Impaired at toes.
2 - Impaired at toes or fingers.

Right ☐ Left ☐

4. Position Sense (test using minimal random movement of distal interphalangeal joints of index finger and big toe)

0 - Normal.
1 - Impaired at toes/or fingers.
2 - Impaired at toes and fingers.

Right ☐ Left ☐

5. DTR (0-absent; 1 -hyporeflexia; 2 -normal; 3 -hyperreflexia; 4 -pathologic hyperreflexia)

Right:
BJ_____ BrJ_____ KJ_____ AJ_____

Left:
BJ_____ BrJ_____ KJ_____ AJ_____

Right Left

0 - No areflexia
1 - Areflexia in either upper or lower limbs.
2 - Generalized areflexia. ☐ ☐

TOTAL PERIFPHERAL 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.

Stance and gait tests may be done barefoot if patient does have appropriate footwear, however, it should be done the same way for serial measurement.)

Circle which: Barefoot Footwear

Also, indicate if AFOs are used: Yes No

1. Sitting Posture (Patient seated in chair with thighs together, arms folded, 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. ☐

2. Stance feet apart– Inside of feet 20 cm apart marked on floor. Use stopwatch; 3 attempts; time in seconds):

   Trial 1 ☐ Trial 2 ☐ Trial 3 ☐ AVG ☐

   0 - 1 minute or longer.
   1 - <1 minute, >45 sec.
   2 - <45 sec., >30 sec.
   3 - <30 sec., >15 sec.
   4 - <15 sec. or needs hands held by assistant/device.

3. Stance - Feet Together (use stopwatch; 3 attempts; time in seconds):

Trial 1 ☐ Trial 2 ☐ Trial 3 ☐ AVG ☐

0 - 1 minute or longer.
1 - <1 minute, >45 sec.
2 - <45 sec., >30 sec.
3 - <30 sec., >15 sec.
4 - <15 sec.

4. Tandem Stance (use stopwatch; 3 attempts, dominant foot in front; time in seconds):

Trial 1 ☐ Trial 2 ☐ Trial 3 ☐ AVG ☐

0 - 1 minute or longer.
1 - <1 minute, >45 sec.
2 - <45 sec., >30 sec.
3 - <30 sec., >15 sec.
4 - <15 sec.

5. Stance on Dominant Foot (use stopwatch; 3 attempts; time in seconds):

Trial 1 ☐ Trial 2 ☐ Trial 3 ☐ AVG ☐

0 - 1 minute or longer.
1 - <1 minute, >45 sec.
2 - <45 sec., >30 sec.
3 - <30 sec., >15 sec.
4 - <15 sec.

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):

0 - Normal (able to tandem walk >8 sequential steps).
1 - Able to tandem walk in < perfect manner/can tandem walk >4 sequential steps, but <8.
2 - Can tandem walk, but fewer than 4 steps before losing balance.
3 - Too poorly coordinated to attempt task.

☐

7. Gait (use stopwatch; walk 8 m/25 ft. at normal pace, turn around using single step pivot and return to start; performed in hallway with no furniture within reach of 1 m/3 ft. and no loose carpet):

Device, if any:_____

Time in seconds:_____

☐

0 - Normal.
1 - Mild ataxia/veering/difficulty in turning; no cane/other support needed to be safe.
2 - Walks with definite ataxia; may need intermittent support/or examiner needs to walk with patient for safety sake.
3 - Moderate ataxia/veering/difficulty in turning; walking requires cane/holding onto examiner with one hand to be safe.
4 - Severe ataxia/veering; walker or both hands of examiner needed.
5 - Cannot walk even with assistance (wheelchair bound).

☐

TOTAL UPRIGHT STABILITY SCORE

TOTAL NEUROLOGIC EXAMINATION SCORE

IV. INSTRUMENTAL TESTING

2. Nine-Hole Pegboard (Make sure the stopwatch is set to zero. Introduce this section by saying, "Now, we're going to be measuring your arm and hand function." If this is the first visit, as, "Are you right- or left-handed?" Make a note of the dominant hand for subsequent instructions. Place the 9-HPT apparatus on the table directly in front of the patient. Arrange the apparatus so that the side with the pegs is in front of the hand being tested and the side with the empty pegboard is in front of the hand not being tested. Secure with Dycem. Read the following instructions to the patient: "On this test, I want you to pick up the pegs one at a time, using one hand only, and put them into the holes as quickly as you can in any order until all the holes are filled. Then, without pausing, remove the pegs one at a time and return them to the container as quickly as you can. We'll have you do this two (2) times with each hand. We'll start with your [DOMINANT] hand. You can hold the peg board steady with you [NON-DOMINANT] hand. If a peg falls onto the table, you retrieve it and continue with the task. If a peg falls on the floor, keep working on the task and I will retrieve it for you. See how fast you can put all the pegs in and take them out again. Are your ready? Begin."

   Start timing as soon as the patient touches the first peg, and stop timing when the last peg hits the container. If a peg drops on the floor, the examiner will retrieve it and put it back in the peg box. However, if a peg drops onto the table, the patient is to retrieve it unless it is beyond their arm reach then you can retrieve it for them. It is possible that a peg may fall beyond the reach of the examiner therefore; we recommend that you keep a few extra pegs in hand so that testing is not interrupted. Do not put extra pegs in the testing apparatus as this may confuse the subject. Record the patient's time under "Dominant hand -- Trial 1." If the subject stops after having put all the pegs into the holes, prompt the subject to move them as well by saying, "And now remove them all." If the subject begins to remove more than one peg at a time, correct him/her by saying, "Pick up one peg at a time."

   The total time to complete the task is recorded in seconds including one decimal place rounded as needed. Round up to the next tenth if hundredth's place is > = .05, round down in hundredth's place is < 0.5.)

	RIGHT		LEFT
Trial 1		Trial 1	
Trial 2		Trial 2	
AVG		AVG	

Attachment II
Protocol Schedule of Event

[a] (+)-EPI drug levels and follistatin/myostatin levels will be analyzed by UC-San Diego. All other laboratory analyses will be conducted by Mayo Clinic Department of Lab Medicine and Pathology and Research Laboratories

15-006845 Protocol Schedule of Events						
Study Activity	**Week 0** Baseline **Clinic Visit**	**Interim Contacts**	**Week 3** (+/- 1 week) **Lab Only**	**Week 12** (+/- 1 week) **Clinic Visit**	**Interim Contacts**	**Week 24** (+/- 1 week) **Clinic Visit**
Visit	**1**			**2**		**3**
Review Eligibility	X					
Informed Consent	X					
Medical History	X			X		X
Concurrent Medications	X			X		X
Physical Exam/Vitals/BP	X			X		X
Drug Level (+)- EPI [a]	X		X	X		X
Follistatin/Myostatin [a]	X		X			X
Blood labs [b]	X			X		X
Urine Analysis [c]	X					X
Neuro Studies [d]	X					X
Cardiac MRI/BOLD	X			X		X
Echo/ECG/Perfusion MRI	X					X
FARS Assessment	X			X		X
Urine Pregnancy Test [e]	X			X		X
Dispense Study Med Dosing/AE Diary [f]	X			X		
Increase Dose, if appl [g]				X		
Review Dosing and AE diary				X		X (collect)
Interim Contacts [h]		X			X	
Off-study evaluation [i]	X					X

[a] (+)-EPI drug levels and follistatin/myostatin levels will be analyzed by UC-San Diego. All other laboratory analyses will be conducted by Mayo Clinic Department of Lab Medicine and Pathology and Research Laboratories

[b]: Serum BNP, Calcium, cardiac troponin I, Complete Blood Count (includes RBCs, WBCs with differential, platelets), ceruloplasmin, creatinine with eGFR, frataxin, glucose tolerance test (not performed week 12), hemoglobin A1C, iron, mitochondrial complex I-V, potassium, PT/PTT, sodium, ST2, triglycerides, liver enzymes (AST, ALT, Alk phos)

[c]: F2-isoprostane

[d]: Magnetic resonance imaging of cerebellum and spinal cord, magnetic resonance spectroscopy

[e]: Urine pregnancy test (females of childbearing potential)

[f]: (+)- EPI orally administered daily for 24 weeks

[g]: (+)-EPI dose will be increased from 75mg/day to 150mg/day, if 8m timed walk via FARS assessment or cardiac evaluation shows no improvement or worsens

[h]: Study site staff will contact the subject/LAR approximately every 15 days between the Baseline, Week 12 and Week 24 visits to assess clinical changes, adverse events and dosing compliance.

[i]. Respiratory chain function, aconitase, catalase, PGC1α, SOD2 in blood if needed