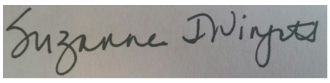

Protocol Title: Antioxidant therapy in RYR1-related congenital myopathy
Abbreviated Title: Antioxidant in RYR1-RM
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Human Research Protections Program Investigator and Staff Training:

"Just in time" human subjects protection training courses are required for investigators and staff participating on this protocol:

(Per SOP 25, PIs or IRBs require investigators and staff working on a protocol to complete the following training, as applicable. Delete those that are not applicable. Indicate "None" if no training beyond the general training is required.)

- Biomedical- Vulnerable Subjects - Research with Children
- Genetic Research in Human Populations
- CITI GCP modules

- International Studies- ICH Overview and ICH- Comparison Between ICH GCP E6 and US FDA Regulations, available to those staff who complete the CITI GCP course
- Unanticipated Problems and Reporting Requirements in Biomedical Research
- Unanticipated Problems and Reporting Requirements in Social and Behavioral Research

Total requested accrual ceilings (separately specify planned accrual for each subject group)
100 Patients (50 in study drug group, 50 in placebo group)
100 Healthy Volunteers

Project Uses Ionizing Radiation: x No □ Yes

IND/IDE □ No xYes (attach FDA documentation)
Drug/Device/#_N-acetylcysteine__125141_____
Sponsor: Dr. Katherine Meilleur__

Durable Power of Attorney X No □ Yes

Multi-institutional Project □ No X Yes
Institution#1__Hospital for Sick Children_____ FWA # FWA00000281__
Date of IRB approval ___6/13/2014_____

(If NIH is the coordinating site, list each institution; attach documentation of IRB approval for each site)

Data and Safety Monitoring Board □No X Yes

Technology Transfer Agreement □ No X Yes
Agreement type and number _MTA with Dr. Dowling/Univ. Toronto NR-MTA-15-001, MTA with Dr. Michael/Hyperion NR-MTA-15-002; CTA with BioAdvantex Pharma NR-CTA-15-001; MTA with Dr. Marks/Columbia Univ. NR-MTA-16-001 (muscle tissue); MTA with Dr. Marks/Columbia Univ. NR-MTA-16-002 (genetic mutations)
____Expiration Date_All: 6 months after termination of protocol _____

Samples are being stored □ No X Yes

Flesch-Kincaid reading level of consent form (exclude boilerplate in assessing reading level):
- Affected Adult consent: 9.0
- Affected 14-17 year old assent: 6.2
- Affected 7-13 year old assent: 5.0
- Healthy volunteers consent: 8.7
- Healthy volunteers 14-17 year old assent: 5.2
- Healthy volunteers 7-13 year old assent: 4.6

Précis:
Although genetic disorders of muscle that present at birth are rare, RYR1-related myopathies comprise the most common non-dystrophic congenital myopathy in the United States, with a prevalence of approximately 1/90,000 people (Amburgey et al, 2011). Causative mutations in the ryanodine receptor gene of skeletal muscle, *RYR1,* have been found in several myopathy subtypes, including central core disease and centronuclear myopathy. These mutations result in defective excitation-contraction coupling and increased mitochondrial oxidative stress. Most patients present in childhood with delayed motor milestones, extremity muscle weakness, impaired ambulation, joint contractures, progressive scoliosis, and in some cases eye movement paralysis, respiratory failure, or susceptibility to malignant hyperthermia, an allelic condition. Despite these important morbidities and the risk of early mortality, ***no treatments exist to date.***

RYR1 encodes a homotetrameric transmembrane ion channel, RyR1, which resides on the terminal sarcoplasmic reticulum in close proximity to the T-tubule. By releasing calcium from the sarcoplasmic reticulum into the cytosol in response to muscle fiber stimulation by the motor neuron at the neuromuscular junction, it mediates excitation-contraction coupling and functions as a regulator of cellular calcium concentrations and redox homeostasis. Dowling et al. (2012) recently elucidated the latter mechanism in zebrafish and patient myotubes, showing that *RYR1* mutations result in increased oxidative stress and that this is rescued in both models by treatment with N-acetylcysteine (NAC), a known anti-oxidant. NAC functions as a precursor of glutathione, an endogenous antioxidant that becomes deficient during oxidative stress. This was substantiated by a cystic fibrosis clinical trial in which low glutathione levels in neutrophils undergoing oxidative stress significantly increased with NAC administration.

Dowling et al. (2012) found significant changes post NAC treatment including increased travel distance (endurance) in zebrafish and complete protection from cell death induced by experimentally increasing oxidative stress in myotubes. Thus NAC was a successful treatment in both ex vivo and in vivo model systems. Based on these results, we plan to perform a randomized, double-blinded, placebo controlled clinical trial of NAC in a subgroup of RYR1-related myopathy patients as the first pathophysiologically based treatment for this devastating disorder.

The **objectives** of the study are to determine if NAC reduces oxidative stress, fatigability, and fatigue in a **study population** of patients with RYR1-RM. The study population includes both males and females 7 years of age and older and age-matched healthy volunteers. The **study design** has two phases. The first 6-month phase will be used to validate the selected outcome measures in RYR1 congenital myopathy. The second 6-month phase is a randomized, double-blinded, placebo controlled drug intervention trial. The primary **outcome measures** are urine 15 F2t-isoprostane for oxidative stress and six minute walk test for fatigability. Healthy volunteers will be evaluated to determine normal values of biomarkers, muscle ultrasound, and near infrared spectroscopy in this rare disease, in order to develop a comparison between healthy and RYR1-RM individuals.

Table of Contents

List of Abbreviations

6MWT: 6 Minute Walk Test
CPET: Cardiopulmonary Exercise Test
DCFH: 2',7'-dichlorofluorescein
DHEA: Dehydroepiandrosterone
EIM: Electrical Impedance Myography
ELISA: enzyme-linked immunosorbent assay
FACIT: Functional Assessment of Chronic Illness Therapy
GSH: glutathione
MFI: Multidimensional Fatigue Inventory
MFM: Motor Function Measure
MRI: Magnetic Resonance Imaging

MRS: Magnetic Resonance Spectroscopy
NAC: N-acetylcysteine
NADPH: Nicotinamide adenine dinucleotide phosphate
NeuroQoL: Quality of Life in Neurological Disorders
NIRS: Near Infrared Spectroscopy
OM: Outcome Measure
PROMIS: Patient-Reported Outcomes Measurement Information System
PTGS2: prostaglandin-dependent cyclo-oxygenase
QoL: Quality of Life
ROS: reactive oxygen species
RYR1: ryanodine receptor 1-related myopathies

1. Introduction

Although genetic disorders of skeletal muscle that present at birth are comparatively rare, RYR1-RM comprises the most common non-dystrophic congenital myopathy in the United States, with a prevalence of approximately 1/90,000 (Amburgey et al., 2011). Causative mutations in the gene (*RYR1)* have been found in several myopathy subtypes, including central core disease and centronuclear myopathy.

The defining histopathological feature of RYR1 mutation on muscle biopsy is the presence of amorphous cores, likely due to reduced oxidative enzyme activity (Jungbluth et al., 2011). Most patients present in childhood with delayed motor milestones, extremity muscle weakness, impaired ambulation, joint contractures, progressive scoliosis, and, in some cases, eye movement paralysis, respiratory failure, or susceptibility to malignant hyperthermia, an allelic condition (Jungbluth et al., 2011; Bevilacqua et al., 2011; Zhou et al., 2007). Despite these important morbidities and the risk of early mortality, ***no treatments exist to date***.

RYR1 encodes a homotetrameric transmembrane ion channel, RyR1, which resides on the terminal sarcoplasmic reticulum in close proximity to the T-tubule. By releasing calcium from the sarcoplasmic reticulum into the cytosol in response to muscle fiber stimulation by the motor neuron at the neuromuscular junction, it mediates excitation-contraction coupling and functions as a regulator of cellular calcium concentrations and redox homeostasis (Bevilacqua et al., 2011, Brookes et al., 2004; Betzenhauser and Marks, 2010). RYR1 gene mutations result in defective excitation-contraction coupling and increased mitochondrial oxidative stress (Jungbluth et al, 2011).

To elucidate novel pathogenic mechanisms in RYR1-RM, Dowling and colleagues (2012) employed expression array analysis in a zebrafish model of RYR1-RM (*ryr* zebrafish), which disclosed abnormalities in pathways associated with cellular stress. Further studies were completed to confirm the presence of increased oxidative stress. Beta-galactosidase, a measure of cellular stress *in vivo*, was elevated in *ryr* zebrafish. *Ryr* zebrafish also showed increased carbonylation by oxyblot, a marker of oxidative stress, compared to wildtype controls (Arbogast et al., 2009). DCFH, a measure of overall intracellular oxidant activity was tested in *ryr* zebrafish against controls and the affected zebrafish showed increased activity. The increased oxidant activity appeared to be a direct result of the *ryr* mutation. Similarly, myotubes from patients with RYR1-RM showed increased oxidative stress (in DCFH assay and oxyblot analysis). Further studies were completed to determine if oxidative stress in RYR1-RM is due to over-abundant oxygen species or to insufficient antioxidants (GSH). Dowling et al. (2012) measured the expression of genes related to antioxidant defense. There were no changes in the expression of the following antioxidant genes: catalase, superoxide dismutase 1, mitochondrial superoxide dismutase and extracellular superoxide dismutase. These results indicate the cause of oxidative stress is not a lack of antioxidants, but rather over-abundance of reactive oxygen species (ROS). Possible sources of reactive oxygen species include NADPH oxidase complex (Hidalgo et al., 2006), PTGS2, and mitochondria. Dowling et al. (2012) tested all three potential sources, yet only mitochondria appeared to be the cause of the over-production of ROS.

Given these findings, Dowling et al. (2012) subsequently tested the effect of NAC in cultured patient myotubes and zebrafish. N-acetylcysteine (NAC) is an antioxidant and a precursor to GSH. By increasing the intracellular content of GSH, NAC augments the cell's reducing potential and restores the redox imbalance (Tirouvanziam et al., 2006). In the experiment, control myotubes and RYR1-related myopathy myotubes were exposed to oxidants,

producing a higher cell death rate in RYR1-RM myotubes. However, when pre-treated with NAC, the RYR1-RM myotubes were completely protected from cell death. *Ryr* zebrafish endurance and swim velocity were also tested. When treated with NAC, *ryr* zebrafish showed rescued endurance (represented by swim distance), matching the endurance of controls treated with NAC. However, strength (represented by swim velocity) did not appear to improve for *ryr* zebrafish treated with NAC. These findings corroborate the known mechanism of NAC, which functions as a precursor of GSH, an endogenous antioxidant that becomes deficient during oxidative stress.

Based on these findings regarding NAC's effect on RYR1-RM in zebrafish and patient myotubes and on evidence of improved oxidative stress (increased GSH) in NAC human trials for other conditions, we plan a clinical proof of concept study. 15-F2t-isoprostane likewise measures oxidative stress (due to lipid peroxidation) and has been reported as the most accurate method to assess *in vivo* oxidative stress status (Milne *et al.* 2012). Early animal studies showed that glutathione peroxidase (GPx), a reducing agent mediated by glutathione availability, may be important for combatting peroxidation of mitochondrial lipid membranes by free radicals (McCay et al. 1976). NAC is a direct precursor to glutathione (Schmitt *et al.* 2015), and RYR1-RM preclinical studies showed that the source of oxidative stress in preclinical disease models was due to mitochondrially derived reactive oxygen species (Dowling et al. 2012).

The treatment group will receive 15mg/kg/day (no more than 1800 mg/day) for the first week. After the first week, subjects who weigh fewer than 50kg will receive 30mg/kg/day of NAC (no more than 2700mg/day) divided TID over a period of six months. To increase compliance, as the tablets are formulated as 900mg each, subjects who weigh greater than 50kg will receive 2700mg/day. This dose is within the range of study references and was approved by the FDA on the IND (125141) for this trial.

This dose is scientifically acceptable. The dose of 2700mg/day is similar to that of other clinical trials. A trial of NAC for cystic fibrosis, using a dose of 0.6 to 1.0g TID, significantly showed increased GSH levels in blood neutrophils. Furthermore, sputum elastase activity, the strongest predictor of CF pulmonary function, markedly decreased ($P = 0.006$) and the treatment was safe (Tirouvanziam et al., 2006). NAC is currently indicated as a mucolytic for cystic fibrosis and other pulmonary conditions. A pediatric study was completed in three to ten year olds with autism to treat behavior issues (Hardan et al, 2012). The dosage began as 900mg once a day for four weeks, and worked up to 900mg TID for four weeks (entire trial was 12 weeks). NAC decreased irritability in the population, measured by the ABC (Aberrant Behavior Checklist) Irritability Score. Side effects were related to GI issues, but there was no significant difference between the side effects of the control group and the treatment group. A trial of NAC in patient with fibrosing alveolitis showed increased GSH levels over a period of 12 weeks (idiopathic pulmonary fibrosis) and is now FDA-approved for this indication (Behr et al, 1997). Natural history data for RYR1-RM are limited to date, but the Motor Function Measure (MFM), a scale that measures motor function, has been validated in this population and has shown stable motor function scores over a 1 year time period (Vuillerot et al., 2012). We intend to use the MFM as a gold-standard outcome measure to track progression of disease in the natural history portion of this trial. Clinical manifestations usually improve in early childhood and may remain stable for some years, as RYR1-RM is not degenerative. However, natural history data observing

oxidative stress levels, fatigability, and fatigue are lacking. Thus, the initial portion of the trial will be a study of the natural history in RyR1-RM.

Summary
A randomized, double-blinded, placebo controlled clinical trial of NAC in RYR1-RM patients will be performed to test the effect on oxidative stress, fatigability, and fatigue. The primary outcome measure will be 15-F2t-isoprostane for oxidative stress. Success will be defined as finding a significant difference in 15-F2t-isoprostane concentration between the treatment and placebo groups at the termination of the study. ***If successful, this study of NAC in RYR1-RM patients will provide the first treatment available for this debilitating muscle disease.***

2. Study objectives

Aim 1. To determine if NAC reduces oxidative stress in patients with RYR1-RM.
Hypothesis: Compared to the placebo group and baseline values for each patient, N-acetylcysteine will reduce oxidative stress in the treatment group as evidenced by: 1) decreased 15-F2t-isoprostane in urine, 2) decreased 2',7'-Dichlorodihydrofluorescein (DCFH) in biopsied muscle, and 3) increased glutathione (GSH) in blood. Exploratory markers of anaerobic threshold, fraction of inspired O2, and tissue oxygenation by Near Infrared Spectroscopy (NIRS) will also be measured pre and post treatment.

Aim 2. To determine if NAC reduces fatigability and fatigue in patients with RYR1-RM.
Hypothesis: Compared to the placebo group and baseline values for each patient, N-acetylcysteine will reduce fatigability (objective construct) and fatigue (subjective construct) in the treatment group as evidenced by: (for fatigability) 1) the 6MWT, 2) timed function tests, 3) grip and pinch strength, as measured by Myotools 4) muscle fatigability by Biodex-; and (for fatigue) 5) Patient-Reported Outcomes Measurement Information System (PROMIS), 6) Functional Assessment Chronic Illness Therapy (FACIT) fatigue scale, 7) Fatigue scales: Multidimensional Fatigue Inventory (MFI).
As secondary and exploratory measures, we will also test the following pre and post treatment: CPET by cycle ergometry, Electrical Impedance Myography (EIM) to measure skeletal muscle health, muscle MRI to evaluate for subclinical muscle involvement as well as the health of muscle tissue, MFM for motor function, salivary fatigue biomarker index (FBI), melatonin, dehydroepiandrosterone (DHEA), the NeuroQoL scale, and qualitative interview for health related quality of life.

Aim 3. To determine values of study biomarkers, NIRS, muscle ultrasound, and MRI in age-matched healthy volunteers

3. Subjects:

a. Description of study populations

This study will include patients age 7 years or older with mutations in *RYR1* (confirmed by previous genetic testing), who are not currently taking antioxidants, and who are able and willing to perform all procedures at the time of enrollment. Children under age 7 may not be

able to successfully follow instructions for outcome measure testing. The accrual ceiling is 100. Withdrawals/dropouts will not be replaced. The target number of completers is 76.

Sixty to 100 age-matched healthy volunteers will be recruited.

NIH employees may participate. The NIH Information Sheet on Staff Research Participation will be provided to any staff member wishing to participate in this study (see Appendix L).

b. Inclusion criteria

Patients:

- Have a confirmed genetic diagnosis of RYR1-related myopathy, including congenital myopathy, OR have a clinical diagnosis of RYR1-related myopathy and a family member with a confirmed genetic diagnosis of RYR1-related myopathy
- Ambulatory.
- 7 years of age or older.
- Preferred: The affected subject has a previous muscle biopsy with available report confirming RYR1-related myopathy histopathology.

Healthy Volunteers:

- Ages 7 and older (15-25 7-12 year olds, 15-25 13-17 year olds, 15-25 18-40 year olds, 15-25 41 and older) without medical condition resulting in or complaints of muscle weakness and/or fatigue.
- The ability to provide consent.

c. Exclusion criteria

Patients:

- Adults who cannot provide their own consent and pediatric participants who do not have a parent able to provide consent
- Patients with a history of liver disease (Liver Function Tests will be collected at baseline and at each study visit as a precautionary measure). Liver disease is defined as moderate to severe hepatic impairment based on the following:

  ALT ≥ 8x upper limit of normal (ULN) with total bilirubin 2x ULN (plus >35% 'direct' bilirubin)and/or INR >1.5

  or

  GGT > 2-3x ULN with bilirubin 2x ULN (plus >35% 'direct' bilirubin) and/or INR >1.5GGT >2-3x ULN and INR >1.5
- Patients with a history of peptic ulcers, gag reflex depression, and esophageal varices. Patients with gastrostomy tubes may be considered for participation, in the case of gag reflex depression or other swallowing or feeding difficulties.
- Patients who have a severe pulmonary dysfunction (FEV1< 40% predicted) or evidence of pulmonary exacerbation. Pulmonary exacerbations refer to an acute worsening of respiratory symptoms that result from a decline in lung function (Flume et al., 2009). Participants may present with increased coughing, increased dyspnea, increased haemoptysis, increased fatigue, decreased pulmonary function by a min of 10%, or a change in sputum color (Bilton et al., 2011).
- Pregnant and breastfeeding women.
- Consumption of antioxidants [including NAC, GSH, melatonin, Immunocal (Immunotac

Research, Vandreuil-Dorion, QC, Canada), Nacystelyn (Galephar, Brussels)] in the 4 weeks before recruitment.

- Daily use of acetaminophen (including Percocet, Vicodin, Oxycodone, Excedrin, and other acetaminophen-containing drugs), nitroglycerine, or carbamazepine during the past 7 days.
- Current use of Angiotensin-converting enzyme (ACE) inhibitors or Angiotensin Receptor Blockers (ARBs)
- Patients who have ever used Beta2-adrenergic agonist tablets, for the purpose of increasing muscle mass (such as albuterol tablets).
- For the muscle biopsy procedure only (second and third visits, if applicable): Patients who have taken Aspirin, Ibuprofen, Advil, Motrin, or Aleve within the 3 days prior to the muscle biopsy procedure, and/or patients who have taken Plavix, fresh garlic, gingko, or ginseng 5 days prior to the muscle biopsy.
- Participation in trials for other therapeutic investigational drugs simultaneously or 4 weeks before recruitment.

Other clinically significant medical disease that, in the judgment of the investigators, would expose the patient to undue risk of harm or prevent the patient from completing the study. Examples include anemia (defined as Hgb < 8 gm/dl), an inability to walk safely without assistance for at least 6 minutes, and/or an inability to consume at least 6 ounces of fluid, 3 times a day, either orally or via G-tube. Patients with comorbidities (i.e. cancer, epilepsy) will be carefully assessed to determine if their comorbidity could lead to confounding or safety issues, should their participation continue.

Healthy Volunteers:

- Diagnosis of RYR1-related myopathy or other neurological disorder (by neurological exam, genetic testing, or muscle biopsy
- Complaints of fatigue or weakness
- Consumption of antioxidants [including NAC, GSH, melatonin, Immunocal (Immunotac
- Research, Vandreuil-Dorion, QC, Canada), Nacystelyn (Galephar, Brussels)] in the 4 weeks before recruitment.
- Use of Beta2-adrenergic agonists.

4. Study Design and Methods:

a. Study overview

The study design has two phases: first, a prospective natural history study and second, prospective, randomized, double-blinded, placebo-controlled phase. Each participant will be in the study for about 18 months from start to finish, with a total of three visits of 4-6 days each and 7 phone calls and/or emails. All emails containing Patient Identifying Information are to be sent using secure email, behind the NIH firewall (SEFT). Participants will be given a link to SEFT and will be asked to send all medical records and other sensitive information through the SEFT system. Visits will be outpatient at the Clinical Center, National Institutes of Health in Bethesda, MD. Samples will be analyzed at National Institutes of Health or at the Eicosanoid Core Facility at Vanderbilt University, Tennessee on a fee-for-service basis. Analyses will be overseen by the Core's Director, Dr. Ginger Milne.

In the natural history phase (Phase 1), participants will be seen at baseline, called one month later (for re-test reliability of fatigue and quality of life scales), and will be seen again at

six months (±2 weeks) to undergo procedures by an investigator trained in questionnaire administration. The participants will complete the scales at home, using CTSS. Sixty to one hundred healthy volunteers will undergo a physical exam, study biomarker, NIRS testing, and muscle ultrasound only, in one outpatient visit to NIH. Eight-32 of these healthy volunteers will also undergo a muscle MRI (2-8 people from each of the 4 age groups. See Inclusion Criteria for age groups of healthy volunteers). Outcome measures are listed and described under "Outcome Measures". Since many outcome measures used in this study are already well established and have been previously used to measure function in other neuromuscular diseases, we will only validate measures novel to RYR1-RM, such as NIRS, biodex fatigue index, and salivary fatigue biomarkers. The self-administered questionnaires will also be validated by analyzing questionnaire data on 20 participants at 0 months and 1 month.

After data collection at six months, for each individual participant, the participant will immediately begin the second phase of the study when they will be randomized, using block randomization, to a placebo or a study drug group at the end of the 6-month visit to NIH. The allocation scheme is computer-generated using random permuted blocks to maintain balance of the two groups (placebo and control) and to maintain balance between children and adults. The participant will be randomized to the study drug or placebo group by an NIH pharmacist, who is blinded to the allocation scheme. Study drug assignments are recorded by the pharmacist, to be revealed only after the study is complete. BioAdvantex Pharma will provide oral effervescent NAC capsules, (PharmaNac) as well as identically packaged placebo capsules so that participants will not be able to distinguish the treatment from placebo. The study drug group will receive 15mg/kg/day (no more than 1800 mg/day) for the first week. After the first week, subjects who weigh fewer than 50kg will receive 30mg/kg/day of NAC (no more than 2700mg/day) divided TID over a period of six months. To increase compliance, as the tablets are formulated as 900mg each, subjects who weigh greater than 50kg will receive 2700mg/day.

After Phase 2 starts, participants will be observed at the NIH for 2 days. They will then receive a phone call and/or email at approximately one and three weeks to monitor their status on the study drug or placebo by a health care professional. After the first month, the subjects will be monitored by phone and/or email monthly (month 2, 3, 4, and 5). Participants will be expected to take their dose three times per day for about six months. Adherence will be assessed by a pill count at their 3-month assessment and at their post-treatment visit (third visit). The pill count will be performed by the participant at home for the 3-month phone call assessment and by a member of our team at NIH during their third visit. Finally, the outcome measures will be obtained at the final visit 6 months (± 2 weeks) after start of treatment (about 12 months into the study). Six months after their third visit (± 2 weeks), the participant will be called for a post-treatment follow up. The participant (or parent) will be asked open-ended questions to assess their symptoms and current medication regime. They will also be asked to fill out the same fatigue and QoL scales completed during their visits, at home on CTSS. The participant will then be finished with the study. Please see Appendix A for a detailed Visits and Procedures Schedule.

b. Recruitment

Although RYR1 congenital myopathies constitute a rare disease, the PIs and AIs on this protocol work closely with patients with this condition and with patient groups such as the Muscular Dystrophy Association and Cure CMD. We will advertise the study through the patient groups by providing information about the study and posting it on their websites (Appendix B). We will

also provide a letter describing the study including contact information to health care providers in the neuromuscular field (Appendix D). If interested, potential participants can call the research coordinator listed on the advertisements.
Flyers have been created for healthy volunteer and patient populations (Appendix B). The patient flyer includes contact information for the Patient Recruitment and Public Liaison office (PRPL). We will distribute our flyers to the managers of the Facebook group pages entitled: "The RYR-1 Foundation", which is found at this link: https://www.facebook.com/ryr1foundation/?fref=ts, and "Central Core Disease & Minicore: a place for support, learning & friends", which is found at this link: https://www.facebook.com/groups/243087794204/. We will also tweet our study at https://twitter.com/NINR and use the web slide carousel on the NINR website https://www.ninr.nih.gov/researchandfunding/dir#.VzzUSRIrKRs, to advertise our study.

A telephone or email prescreening will be used to determine preliminary eligibility (prescreen questionnaire, Appendix G). The prescreening telephone call will be scheduled or the prescreen questions will be sent via email. During the prescreening phase, we will request the potential participant send the results of their genetic testing confirming diagnosis of RYR1 (if done) and the results of their muscle biopsy confirming RYR1 histopathology (if done). We will receive these documents via mail, fax, or secure email before their visit. This will prevent patients who are clearly not eligible from making an unnecessary trip to NIH. The records of any participant who subsequently does not qualify will be destroyed or returned to the participant.

We will not request records of relatives of participants/potential participants who are enrolling under the criteria of a clinical diagnosis with a family member with confirmed RYR1.

There is no direct solicitation of employees/staff by supervisors. Direct solicitation by co-workers is considered improper.

c. Screening

Patients:

Informed consent will be obtained before any study procedures begin. We will screen participants by determining whether or not they meet inclusion/exclusion criteria by using the eligibility checklist (Appendix E).

At the onset of the study, individuals will be required to have a documented muscle biopsy confirming RYR1 diagnosis. If 20 individuals with this documentation cannot be recruited within the first 6 months, those without muscle biopsy confirmation will also be enrolled.

Healthy Volunteers:

Healthy volunteers will be screened by phone, email, or in-person for the presence of any neurological condition and/or complaints of weakness and fatigue and will be asked about current medications, per the eligibility criteria.

d. Study procedures

Patients:

Participants will travel to the NIH Clinical Center three times over the course of one year. At each visit, participants will complete some or all of the outcome measure tests and up to 20 adults who volunteer will undergo optional muscle biopsy testing at the second and third visit for

a maximum of two biopsies. The muscle biopsy is the only procedure that will not be required of all participants due to its invasive nature, all other procedures are required unless contraindicated.

All study procedures are for research purposes and consent will be obtained prior to any procedures. No radiation will be used in the study. All visits are part of the study. Participants will follow up with their primary physician or neurologist for clinical care.

Medical History: Participants will have access to an encrypted online survey system (Clinical Trial Survey System or CTSS) to enter their coded medical history information. Participants will be given the website and login by an investigator. Legal guardians may enter this information for minors. Study physicians, nurse practitioners and/or nurses will obtain medical history information to supplement the online medical history if needed.

Physical Examination: Height, weight, head circumference, BMI, and ulna length may be obtained by trained staff, including nurses, nurse practitioners, physicians, and physical therapists. Height, ulnar length, and head circumference will be repeated in children only at visit 2 and 3. For participants with contractures, height may be estimated by ulnar length obtained by a segmometer, which has been shown to correlate with height. If available, a sling scale will be used to weigh participants who are unable to stand on a scale. Study physicians or nurse practitioners will perform a physical examination including the evaluation of cranial nerves, reflexes, sensation, strength, gait, movement, motor function and coordination. The physical examination will take approximately 30 minutes.

Cardiology: Cardiologic evaluations in individuals will include electrocardiogram (EKG), which is required prior to CPET. EKGs take 10 minutes or less. A cardiology consult will be requested only if the EKG is abnormal. Participants deemed unable to complete the CPET will not be excluded from the rest of the study procedures.

Pulmonary Functions Tests: Each subject will perform pulmonary function testing (PFTs) to assess vital capacity (VC) and maximal voluntary ventilation (MVV). To measure VC the participant will be asked to take the deepest breath they can, and then exhale into a flow sensor. For maximal voluntary ventilation (MVV) the patient will be instructed to breathe as fast and deep as possible for 12 seconds. During the test, soft nose clips may be used to prevent air escaping through the nose (patients will use their own flow sensors so filters will not be needed). Measures to be obtained include vital capacity (VC), forced expiratory volume at 1 second (FEV1), and maximal voluntary ventilation. Pulmonary Function testing will take approximately 30 minutes.

Cardiopulmonary Exercise Testing (CPET): The cardiopulmonary exercise test (CPET) will be administered using an electronically braked cycle ergometer. Subjects will be asked to maintain a pedaling cadence of at least 30 revolutions per minute (rpm) for the entire test. Subjects will begin the CPET pedaling against an unloaded work rate for the first minute after which point the work rate will increase each additional minute. The CPET will be stopped when the subject can no longer maintain the predetermined pedaling cadence despite strong verbal encouragement. Test duration, maximal oxygen uptake (VO_{2max}), and Anaerobic Threshold, determined using the

V-slope method, will be obtained during CPET testing. CPET takes approximately 1 hour to complete.

Electrical Impedance Myography (EIM) Assessment: Measurements of electrical impedance of muscle tissue will be made to determine the health of the muscles by using an electrical impedance investigational device. Current and sensing electrodes are placed non-invasively on the subject's skin at known distances and the voltage at the sensing electrode is measured. Resistance, reactance, and the phase of multiple muscles are then recorded and the interpretation of these results is used to determine the health of the muscle tissue.

Magnetic resonance imaging (MRI): An MRI scan of the leg muscles may be performed in order to visualize structures using a 1.5 or 3 Tesla Magnet. T2 and T1 weighted imaging and MRS will be performed. High resolution conventional MRI will be performed with volumetric reconstruction of designated regions. Time in the MRI scanner will be limited to 1 hour, in order to allow the subject to rest and assure the best quality of the images. We will allot additional time for any delays and to better ensure comfort. Audiovisual distraction equipment such as music system with headphones will be available. Children will be given extra time to get adjusted to the new environment in the imaging facility, as well as in the scan room to alleviate anxiety and nervousness. Sedation will not be used for any procedure.

Arterial Occlusion Muscle Oxygenation Capacity Test: A blood pressure cuff will be placed around the distal thigh of the dominant leg. A near infrared spectroscopy (NIRS) light source and receiving sensors will be placed on the leg, distal to the occlusion. Using a Hokanson® rapid inflation vascular testing system, the cuff will be rapidly inflated to a pressure that totally occludes blood flow into the gastrocnemius, typically between 60 and 80 mmHg above resting systolic pressure. The occlusion will be maintained for up to 10 minutes and tissue saturation will be measured over the time course. The tissue saturation index will be calculated as TOI= [O2-Hb] / Tot-Hb. The rate of decline and rise in TOI will be the main muscle tissue saturation measures.

Physical therapy: Evaluation will include timed functional tests: 10 meter walk test, supine to stand, ascend 4 stairs, descend 4 stairs, and the 6 minute walk test. Grip and pinch strength will also be determined with Myotools. The myogrip is a hand held dynamometer designed to measure grip strength, while the myopinch is a pinch gauge designed to measure finger strength. The patient will be seated comfortably with his/her elbow flexed to 90 degrees, with the forearm and wrist in neutral position. The patient is asked to squeeze the dynamometer and pinch the gauge. This process is repeated three times (3 trials). Time to complete: 30 min.

Motor Function Measure 32 (MFM): The MFM is a generic scale which provides a measurement of the effects of muscle weakness in neuromuscular diseases (NMD). Assessments are based on posture and movements of the whole body. It is applicable to both ambulant and non-ambulant patients with a wide range of disease severity. The patient is asked to roll, sit, lift head from prone and supine position, get up from a lying position, prop on arms, kneel, crawl, stand and step. This test will be combined with timed tests (floor to stand, ascend/descend 4 steps). Time to complete both tests: approximately 60 minutes.

6 Minute Walk (6MWT): The self-paced 6MWT assesses the submaximal level of functional capacity. The patient is asked to walk for 6 minutes with their best effort. A clinician accompanies the patient during the test to ensure patient's safety. Heart rate and blood pressure is monitored before and after exercise and after 5 minutes of recovery to ensure safety of the patient. The patient is allowed to stop if he feels tired. Time to complete the entire test: approximately 20 minutes.

Biodex: Biodex will be used to test muscle groups using a dynamic sub-max fatigue test with a repetitive motion. Blood pressure will be taken prior to testing to confirm the patient is not hypertensive (>140/90). Clinicians will assess participants for previous knee injury prior to testing. The biodex test will not be performed if the participant has a history of knee injury. Clinicians will provide a 5-minute break immediately after the test, during which time the patient may not rise from the seat. Time to complete the entire test: approximately 60 minutes.

Phlebotomy, Genetic Testing, and Exome Sequencing: Phlebotomy will be performed to obtain blood for liver function testing at baseline and glutathione levels. Blood and blood products may be analyzed or stored for creatine kinase (CK) level, lactate/pyruvate ratio, cell counts, blood chemistries, molecular genetic analysis and DNA banking. The lab will draw blood for DNA to confirm a genetic mutation, for exome sequencing, and/or for future studies and other clinical lab testing. If confirmatory genetic testing of an enrolled family member reveals that a participant does not have an *RYR1* mutation causative of RYR1-RM, the participant will be informed and withdrawn from the study.
DNA studies may increase knowledge of the pathomechanism underlying RYR1. The target blood draw is 30ml. A local anesthetic cream such as Eutectic Mixture of Local Anesthetics (EMLA) may be used to minimize the pain for young children. Phlebotomy will take approximately 15 minutes. Blood will not be drawn on participants if they are known to be anemic (Hgb < 8 gm/dl) per medical history, or if their medical condition causes an investigator to believe that blood drawing might be detrimental to the participant. For pediatric participants, no more than 5 mL/kg will be drawn in a single day, and no more than 9.5 mL/kg will be drawn over any eight-week period. Timing of blood draw will be between 8:30-10:30am and will be taken prior to any food consumption. Participants will be notified the night before not to consume food prior to the blood draw the next day.

Saliva collection: For the saliva collection, patients will be asked to chew on a cotton swab for about 5 minutes and then deposit the cotton swab in a specimen container. Saliva will be measured for exploratory salivary biomarkers of fatigue such as fatigue biomarker index, melatonin, cortisol, and didehydroepiandrosterone (DHEA). Saliva will be collected the same day as the blood draw by a health care professional between 8:30 and 10:30 am prior to any food consumption. Participants will be notified the night before not to consume food prior to the saliva collection the next day. Also, a non-fasting post-exertion saliva sample will be taken immediately after the participant performs the biodex test. The saliva will be put on ice immediately and stored at -80.

Urine collection: Participants will be asked to provide a clean catch urine sample of at least 10 ml. Urine collection is required to detect levels of 15-isoprostane-F2 (normalized for creatinine), which allows for the indirect measurement of ROS. Additionally, urine samples will be used for pregnancy tests.

Pregnancy testing: All women and girls of childbearing potential will have a urine pregnancy test performed at each visit, which must be negative before proceeding further with the study. Contraception will be required for women and girls of childbearing age who are sexually active during phase 2 of the study. If a pregnancy test is positive, the study participant will be notified of the results. If the study participant is a minor, she will be notified, and we will help her inform her parent(s) or guardian of the results. Women who become pregnant during the study will be followed through their pregnancy and birth.

Videotaping and/or Photography: Videotaping or photography may be performed during portions of the physical examination to accurately document the condition. When possible, the patient's face and other identifying marks will not be included in the recording or photograph in order to protect confidentiality. Consent will be obtained for videotaping and photography.

Qualitative interviews and/or questionnaires of fatigue and quality of life: Questionnaires to be used include Neuromuscular Quality of Life (NeuroQOL), the Patient-Reported Outcomes Measurement Information System (PROMIS), FACIT (Functional Assessment of Chronic Illness Therapy), and MFI (Multidimensional Fatigue Inventory). Patients and/or parents will be asked to participate in research qualitative interviews. Parent proxy measures and patient outcome measures will be used to measure patient quality of life and daily function. Interviews and questionnaires will take approximately 2 hours. The NeuroQOL, PROMIS, FACIT, and MFI will be readministered at 1 month after the initial visit and will be completed by the participant in CTSS. Coded questionnaires may be administered online in a database such as CTSS or Assessment Center Lite.

Muscle Ultrasound: Ultrasound will be used to evaluate skeletal muscles using standard medical soft tissue ultrasound. Muscle ultrasound will take approximately 30 minutes.

Muscle biopsy (OPTIONAL, Adults Only): A needle muscle biopsy will be obtained twice during the study on up to 20 adults who voluntarily comply with this procedure at 6 months and 12 months into the study. An unblinded researcher, independent of Dr. Meilleur's research team, will allocate an approximately equal number of subjects from each arm of the study based on the randomization scheme created for the entire study in advance by the NIH pharmacy for muscle biopsy: approximately 10 subjects from the intervention arm will be biopsied, approximately 10 subjects from the placebo arm will be biopsied. An outside muscle biopsy performed by the participants' local providers in the six months prior to the study 6-month time point will also be acceptable, to reduce the number of invasive procedures.

This muscle biopsy will be performed on the vastus lateralis or tibialis anterior muscle. After skin preparation using povidone-iodine or equivalent antiseptic, the skin and subcutaneous tissues will be anesthetized with 7-10 ml of 1% lidocaine without epinephrine. For side-cut biopsy needles (known as Bergstrom or UCHL needles) a 3mm incision will be made in the skin and muscle fascia, and muscle tissue will be obtained with 1 to 3 successful passes of the biopsy needle. Successful passes are defined as passes which obtain any muscle tissue. Due to issues, such as fatty infiltration of muscle in RYR1-RM, one additional incision may be made to obtain muscle tissue. The total number of passes will be limited to 6. Biopsy specimens (5 to 40 mg)

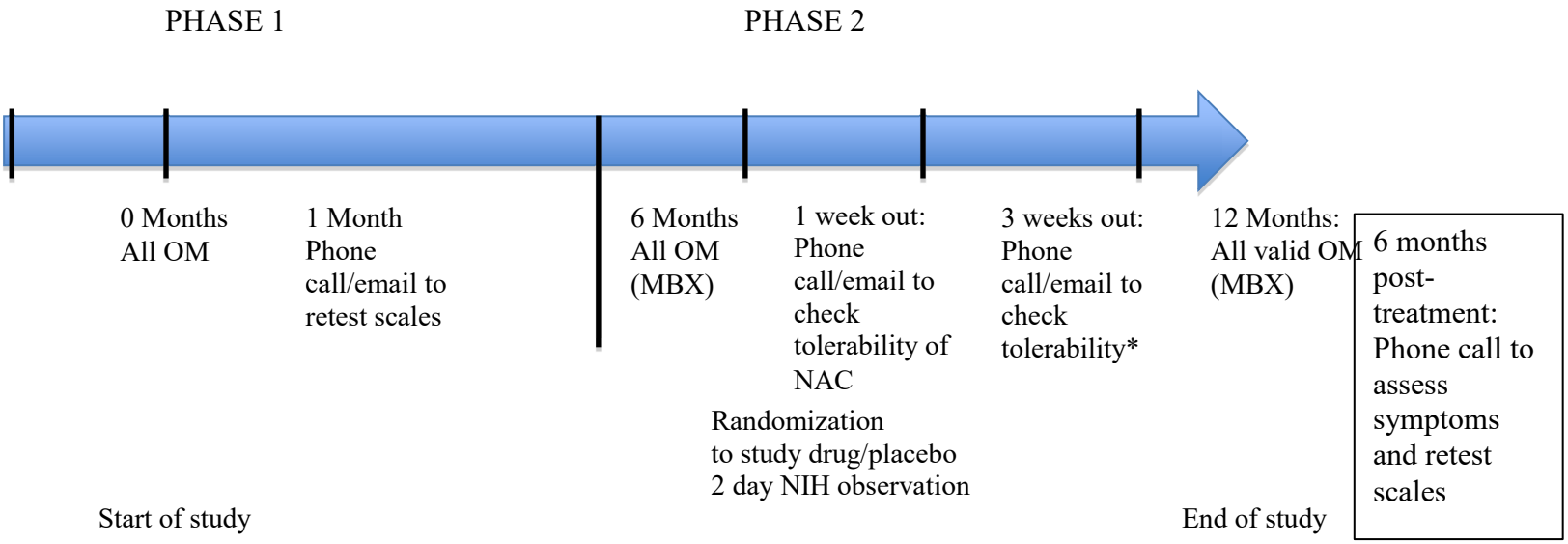
will be frozen as 1 or 2 aliquots depending on tissue yield. The aliquots will be frozen in liquid nitrogen within 2 minutes of removal and stored at -80°C until use. If additional tissue is obtained, it will be prepared as primary muscle cells and/or cell lines. Patients will be evaluated on the last day of the study visit to ask about post-biopsy pain or complications. To minimize variance resulting from time of day or muscle activity, the biopsy will be performed at a similar time of day and after a similar schedule of activities in all subjects at both study visits. Participants will be provided with a "Needle Aspiration Muscle Biopsy Procedure Information Sheet" (Appendix I.) prior to their visit, which describes the pre-procure medication restrictions, procedure details, and post-procedure care instructions. This muscle biopsy will not be performed in minors.

Administration of study drug: Before participants return for their second visit, they (and/or their parent) will be provided with a "Medication/Placebo Information and Instructions Sheet" (Appendix J). This sheet provides information about the drug, dosages, side effects, and contact information. The study drug group will receive 15mg/kg /day (no more than 1800 mg/day) for the first week. After the first week, subjects who weigh fewer than 50kg will receive 30mg/kg/day of NAC (no more than 2700mg/day) divided TID over a period of six months. To increase compliance, as the tablets are formulated as 900mg each, subjects who weigh greater than 50kg will receive 2700mg/day. After the initial dose, participants will have two outpatient clinic visits with vital signs at the NIH. The first clinic visit will include 2 hours of observation after the first dose is administered. The second clinic visit will be the following day and will also include vitals.

Phone calls and emails: Participants will be called or emailed one month after their first visit as a reminder to complete the FACIT, MFI, NeuroQoL, and PROMIS scales. Additional phone calls and/or emails will occur 1 week, 3 weeks and then monthly starting at 2 months after the placebo or drug is started to check for tolerability.

Pill count: Participants will be instructed to count their pills and give the number of unused pills to the licensed care provider, during their 3-month post intervention assessment. This number will be compared to their expected number of remaining pills. Participants will also be instructed to save all leftover pills and bring them to their post-intervention (third) visit. During that visit, the leftover pills will be counted and this number will be compared to the expected number of remaining pills. All participants should have some remaining pills as all are given extra pills, as a precaution. If it is discovered that a participant has missed >20% of their expected number of doses during their 3-month post intervention assessment, the participant will be informed that they need to increase their compliance, in order to remain in the study. If it is discovered that a participant has missed >20% of their expected number of doses after returning for their third visit, the participant will be withdrawn from the study due to non-compliance.

The overall study flow is depicted in the diagram below.



OM-Outcome measures, MBX=Muscle biopsy on select adults
*Participants will then be phoned or emailed monthly after the start of Phase 2

Healthy Volunteers:
Phase 1 only

Physical Examination: Height, weight, head circumference, BMI, and ulna length may be obtained by trained staff, including nurses, nurse practitioners, physicians, and physical therapists. Study physicians or nurse practitioners will perform a physical examination including the evaluation of cranial nerves, reflexes, sensation, strength, gait, movement, motor function and coordination. The physical examination will take approximately 30 minutes.

Videotaping and/or Photography: Videotaping or photography may be performed during portions of the physical examination. When possible, the participant's face and other identifying marks will not be included in the recording or photograph in order to protect confidentiality. Consent will be obtained for videotaping and photography.

Phlebotomy: Phlebotomy will be performed to obtain blood for liver function testing at baseline and glutathione levels. Blood and blood products may be analyzed or stored for creatine kinase (CK) level, lactate/pyruvate ratio, cell counts, blood chemistries. The target blood draw is 30ml. A local anesthetic cream such as Eutectic Mixture of Local Anesthetics (EMLA) may be used to minimize the pain for young children. Phlebotomy will take approximately 15 minutes. Blood will not be drawn on participants if they are known to be anemic (Hgb < 8 gm/dl) per medical history, or if their medical condition causes an investigator to believe that blood drawing might be

detrimental to the participant. For pediatric participants, no more than 5 mL/kg will be drawn in a single day, and no more than 9.5 mL/kg will be drawn over any eight-week period. Timing of blood draw will be between 8:30-10:30am and will be taken prior to any food consumption. Participants will be notified the night before not to consume food prior to the blood draw the next day.

Saliva collection: For the saliva collection, participants will be asked to chew on a cotton swab for about 5 minutes and then deposit the cotton swab in a specimen container. Saliva will be measured for exploratory salivary biomarkers of fatigue such as fatigue biomarker index, melatonin, cortisol, and didehydroepiandrosterone (DHEA). Saliva will be collected the same day as the blood draw by a health care professional between 8:30 and 10:30 am prior to any food consumption. Participants will be notified the night before not to consume food prior to the saliva collection the next day. The saliva will be put on ice immediately and stored at -80.

Urine collection: Participants will be asked to provide a clean catch urine sample of at least 10 ml. Urine collection is required to detect levels of 15-isoprostane-F2, which allows for the indirect measurement of ROS.

Arterial Occlusion Muscle Oxygenation Capacity Test: A blood pressure cuff will be placed around the distal thigh of the dominant leg. A near infrared spectroscopy (NIRS) light source and receiving sensors will be placed on the leg, distal to the occlusion. Using a Hokanson® rapid inflation vascular testing system, the cuff will be rapidly inflated to a pressure that totally occludes blood flow into the gastrocnemius, typically between 60 and 80 mmHg above resting systolic pressure. The occlusion will be maintained for up to 10 minutes and tissue saturation will be measured over the time course. The tissue saturation index will be calculated as TOI= [O2-Hb] / Tot-Hb. The rate of decline and rise in TOI will be the main muscle tissue saturation measures.

Muscle Ultrasound: Ultrasound will be used to evaluate skeletal muscles using standard medical soft tissue ultrasound. Muscle ultrasound will take approximately 30 minutes

Magnetic resonance imaging (MRI): Only 2-8 people from each of the 4 age groups will be asked to undergo an MRI. An MRI scan of the leg muscles may be performed in order to visualize structures using a 1.5 or 3 Tesla Magnet. T2 and T1 weighted imaging and MRS will be performed. High resolution conventional MRI will be performed with volumetric reconstruction of designated regions. Time in the MRI scanner will be limited to 1 hour, in order to allow the subject to rest and assure the best quality of the images. We will allot additional time for any delays and to better ensure comfort. Audiovisual distraction equipment such as music system with headphones will be available. Children will be given extra time to get adjusted to the new environment in the imaging facility, as well as in the scan room to alleviate anxiety and nervousness. Sedation will not be used for any procedure.

e. End of participation
Patients:

Participants will remain under the care of their local physicians and/or primary neurologist while under this study. Participants will receive the results of any clinically relevant findings during the study.

Healthy Volunteers:

Healthy volunteers will have one visit only during Phase 1 and then their participation will be complete.

5. Storage of Data and Samples

Patients:

If permitted by the participants in the consent process, samples and data will be retained, including samples (blood, DNA, urine) and data (results of rehabilitation medicine Department testing, questionnaires, and qualitative interviews). This protocol is subject to the Genomic Data Sharing Policy. De-identified exome data will be submitted to a database (to be decided) through unrestricted access within 1 year of obtaining the data. Exome data will be obtained by sending de-identified DNA samples to a fee-for-service company to perform the exome sequencing. To begin, we will send up to 10 exomes for exome sequencing. The location of storage for the samples will be the laboratory of the National Institute of Nursing Research. Data will be stored in the offices of Dr. Meilleur's team on the NIH campus.. De-identified samples will be sent to Emory University for analysis only and will not be stored at Emory. MTAs have been obtained to transfer coded muscle tissue samples and coded genetic mutations to Dr. Andy Marks at Columbia University. Mutations identified by exome sequencing will be shared with Dr. Andy Marks (NR-MTA-16-002) by sending an excel file by SEFT to him and/or designated his lab members. A Certificate of Confidentiality is not needed.

Samples will be sent to Dr. Milne at Vanderbilt University and Dr. Tirouvanziam at Emory University for analysis on a fee-for-service basis. These entities may also assist in interpretation of de-identified data for their respective analyses. Samples and data will be shared with Dr. Michael at Hyperion (non-genomic data) and Dr. Andy Marks at Columbia University (including genomic data).

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Analyses at Vanderbilt and Emory Universities will be conducted on a fee-for-service basis. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review.

Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

Healthy Volunteers:
If permitted by the participants in the consent process, samples and data will be retained, including samples (blood, urine, saliva). The location of storage for the samples will be the laboratory of the National Institute of Nursing Research. Data will be stored in the office of Dr. Meilleur on the NIH campus. De-identified samples will be sent to Vanderbilt and Emory Universities for analysis and will not be stored at either site.

6. Additional Considerations

a. Research with investigational drugs or devices

N-acetylcysteine (NAC) requires an IND, and the FDA approved IND number is: 125141. The company providing NAC is BioAdvantex Pharma. The study sponsor is Dr. Katherine Meilleur at NIH.

7. Risks and Discomforts

Medical history: There may be some psychological distress associated with providing medical and family history. Frequent breaks or even cessation of procedure may be taken to reduce risk of distress.

Physical examination: There is minimal medical risk and discomfort from the physical examination. This risk also applies to healthy volunteers.

Cardiology evaluation: There are no known complications associated with a cardiology consult or the use of echocardiography. Patients may experience slight discomfort from laying still. There are no known risks for the EKG, however patients may experience discomfort with removal of the electrodes or a topical reaction to the adhesive on the electrodes. There are no known risks for Holter monitoring however prolonged application of the adhesive electrode patches may cause skin breakdown irritation at the application site.

Pulmonary Function Tests: There minimal risks. Participants may feel tired after the procedures.

CPET: Decreased risk when screened and monitored with 12 lead EKG. 1/20,000 deaths in adults with cardiac disease (Paridon et al., 2006). There is literature to suggest that patients with mutations in RYR1 are susceptible to exertional rhabdomyolysis or heatstroke with extreme exercise. We will not be having the patients do extreme exercise, but will take precautions to be ready to prevent this. See “Subject Monitoring” for further details.

EIM: This procedure is non-invasive, but there may be slight discomfort (pressure) with the placement of the electrode on the skin.

MRI: Participants are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Individuals will be screened for these conditions before having any scan, and if they have any, they will not receive an MRI scan. In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room. This risk also applies to healthy volunteers.

Participants with fear of confined spaces may become anxious during an MRI. Those with back problems or significant contractures may have pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in participants who already have hearing loss. Everyone having a MRI scan will be fitted with hearing protection. There are no known long-term risks of MRI scans. Participants may learn pregnancy status through required pregnancy testing.

Physical therapy: 6 Minute Walk, Motor Function Measure32, timed tests, and Myotools tests are patient self-limiting tests with minimal risk and discomfort. Participants may become fatigued.

Biodex: Biodex may produce strain on the heart, muscles, and joints. The following contraindications will be ruled out prior to proceeding with Biodex: history of CAD and/or valvular disease, hypertension (>140/90), aneurysm, severe osteoporosis (BMD >-2.5 standard deviations and history of pathological fracture), acute sprain or strain, unstable joint/bone, knee pain, quadriceps tendonitis, moderate-severe joint effusion, extremely limited joint range of motion, severe pain, soft tissue healing constraints, unable to follow commands appropriately, unable to perform desired motion. BP would be taken immediately prior to testing to confirm appropriate parameters.

Arterial Occlusion Muscle Oxygenation Capacity Test: The only known risk associated with arterial occlusion is discomfort. This procedure will not be performed on individuals who have disorders predisposing them to thrombosis, as a precaution. Arterial occlusion has been performed in patients with various myopathies, including Duchenne muscular dystrophy, and there were no reported adverse events (Sander et al., 2000). There are no published data to our knowledge regarding the incidence of a clot being formed or dislodged due to this procedure. However, due to the nature of the procedure, this is a theoretical risk. Thus, we are excluding subjects with disorders predisposing them to thrombosis, as a precaution. This risk also applies to healthy volunteers.

Phlebotomy: Participants may have some discomfort and bruising at the site of needle entry. There is a very small risk of fainting. Infection in the area of the needle insertion is rare. This risk also applies to healthy volunteers.

Genetic Testing: Risks of sharing genomic data may include the possibility of re-identification.

Saliva collection: There are minimal risks associated with saliva collection. This also applies to healthy volunteers.

Urine collection: There are minimal known risks associated with urine collection. This also applies to healthy volunteers.

Urine pregnancy test: there is a risk that a pregnancy test will be positive and this will be upsetting to the participant or the parent of the participant, if a minor. A clinician will be present the results to the participant and be available for any questions.

Photographs/videos: Risks are minimal. The patient may be identified if a face is featured. When possible, the patient's face and other identifying marks will not be included in the recording or photograph in order to protect confidentiality. This also applies to healthy volunteers.

Qualitative interviews and/or questionnaires: The interviews and questionnaires are not harmful, but they may be frustrating or stressful. There may be some psychological distress associated with answering personal questions. None of the questionnaires query thoughts of harm to self or others. However, some of the questions ask the participants about depression, anxiety and anger and these responses may not be viewed by the investigators for several days. If upon review of the answers, the investigators feel there exists a possibility of a psychiatric diagnosis, the adult or parent, in the case of a minor, will be contacted in order to inform them of the possibility and they will be given a referral to a psychiatric professional in their area.

Muscle ultrasound: Muscle ultrasound is not harmful. Participants may experience some slight discomfort from having to stay still during the procedure or may experience discomfort from the coldness of the gel. This risk also applies to healthy volunteers.

Muscle biopsy: The muscle punch-biopsy procedure has potential risks including infection, bleeding, pain, and post-procedure anesthesia or paresthesia if a cutaneous nerve is damaged. The risk of these events is minimized by having the procedure performed only by experienced neuromuscular licensed care providers under sterile conditions with adequate local anesthesia.

Drug Administration: Please see next section.

Phone calls and email correspondence: Minimal risk.

Pill count: Minimal risk.

Provide the incidence/frequency of adverse effects, if known

When administered as oral effervescent tablets, NAC has few severe side effects, providing a favorable risk benefit ratio to the proposed trial (Tirouvanziam et al, 2006; Hardan et al., 2012). Side effects of PharmaNAC effervescent tablets, which we plan to administer, include diarrhea, nausea, abdominal pain, vomiting, constipation, and flatulence, particularly when used chronically in high doses up to 6.9g/day. The dosage (2700mg/day) for our study is within the

range of dosages previously used in clinical trials and is considered high. If the participants, at any point in the study, should need to take antibiotics, they will be informed to take their antibiotic medication (penicillins, tetracyclines, cephalosporins, and aminoglycosides) at least two hours prior to taking NAC. Other drugs that interact with NAC and GSH include, nitroglycerine, carbamazepine for NAC, and acetaminophen for GSH. Use of these medications is exclusionary, and participants cannot have taken them within seven days of the start of the study. Participants will not stop taking prescription medications for the purposes of this study unless they can be safely discontinued. In this case, the discontinuation will be done under the care of the prescribing physician. It is important to note that high levels of NAC administration have not been tested in pregnant or lactating women, therefore, pregnancy is an exclusion criteria. As mentioned previously, if women become pregnant during this study, we will follow up with these women during their pregnancy and at birth.

Side effects reported when NAC is inhaled or injected include rhinorrhea, stomatitis, generalized urticarial/facial flushing, rash, pruritis, headache, drowsiness, clamminess, chest tightness, bronchoconstriction, and hypotension. Anaphylaxis is a rare but possible side effect in IV administered NAC in response to acetaminophen overdose. However, we will not be administering NAC in the inhaled or IV routes, and there have been no reports of anaphylaxis with oral administration.

Reported side effects for FDA-approved prescription medications differ from those reported for over-the-counter oral supplements. For example, the FDA-approved intravenous formulation of NAC has a side effect of prolonged bleeding (delayed blood clotting). However, this side effect has not been reported for the dietary supplement of NAC. Other side effects related to prescription formulations of NAC, especially those that are intravenous, inhaled or injected, may be found in the literature and on the internet, but may not be applicable to the dietary supplement utilized in this study. Participants are informed that they may find information about side effects related to NAC, which may in fact only be related to other prescription formulations of the drug. Participants are expected to inform study investigators about any concerns they have regarding side effects for NAC found in outside sources.

In a pediatric study by Tirouvanziam et al. (2006), 18 patients took the approximate dose in our proposed study using the same administration we plan to use (PharmaNAC effervescent tablets). 7/18 of these patients had mild symptoms of heartburn (4 patients), bad taste (1 patient), flatulence (1 patient), and nausea (1 patient). None of these symptoms required clinical monitoring. In another pediatric study by Hardan et al (2012) also using the PharmaNAC effervescent tablets, but through a higher dose range, thirty-three subjects (31 male subjects, 2 female subjects; **aged 3.2–10.7 years**) were randomized to receive NAC or placebo for 12 weeks. Subjects were initiated on NAC at **900 mg daily for 4 weeks, then 900 mg twice daily for 4 weeks and 900 mg three times daily for 4 weeks**. This was a 12-week double blind, randomized, placebo-controlled trial in children with autistic disorder. Based on the result of the study, NAC resulted in significant improvements on an irritability subscale compared to placebo. The most common adverse effects observed during the study were gastrointestinal and included nausea/vomiting, constipation, increased/decreased appetite and diarrhea (Hardan et al., 2012). We expect the same side effects in our pediatric population given that NAC is a precursor to the endogenous antioxidant GSH. We do not suspect any specific side effects in myopathies given this mechanism of action.

During the course of this study, one participant experienced rash and chest tightness with coughing, one participant experienced hives, one participant experienced tachycardia, two

participants experienced hypotension, and some participants experienced dehydration, dizziness, and/or headaches. One participant had a benign ovarian cyst, which grew during the course of the study, including during the drug phase. It is not known if these participants were on NAC or placebo.

Steps taken to minimize risk

In our proposed study, after participants receive drug or placebo effervescent tablets at the 6-month visit, they will be monitored at NIH for 2 consecutive days as an extra precaution to rule out rhinorrhea, stomatitis, urticarial/facial flushing, rash, pruritus, hives, coughing, headache, drowsiness, clamminess, chest tightness, bronchoconstriction, and hypotension, which are the most severe potential side effects of inhaled or injected NAC. This monitoring will include being observed in the outpatient clinic for 2 hours after their initial dose, including having vitals signs measured. The second day of observation at NIH will include an outpatient clinic visit with vital signs. If at any other time, the participant notices any symptoms, they will be directed to contact our onsite nurses. A Licensed Care Provider will then call them at one and three weeks to monitor adverse reactions. We will then monitor participants monthly (2, 3, 4, and 5 months) by phone and/or email, primarily for gastrointestinal symptoms, which are the main symptoms of PharmaNAC effervescent tablets. If a patient reports any of the side effects that have already been reported (rash, chest tightness with coughing, hives, or hypotension), they will be advised to stop taking the study medication immediately. They will only restart the study medication after discussing their symptoms with the PI. Patients are also asked, in the consent form, to limit their alcohol consumption to no more than 2 alcoholic drinks per day as excess alcholol consumption may interact with glutathione levels. Participants are informed to call study investigators immediately if they have any problems or side effects and are provided with contact information. They are instructed to visit the emergency room if they have a possible allergic reaction or a serious or life-threatening emergency. They are expected to tell the ER staff that they are in a research study and ask the emergency personnel call the research nurse. Participants are instructed to continue under the care of their local clinicians throughout participation. Participants are provided with a "NAC Information Letter for Clinicians" (Appendix F) to share with their home clinicians. This letter provides study drug information and information about possible side effects.

If a patient reports Grade 3 side effects for diarrhea, nausea, vomiting, constipation, or abdominal pain, or any other Grade 3 side effect, according to the NCI Common Terminology Criteria for Adverse Events v.3.0, we will discontinue their participation. If Grade 1 side effects are reported, we will monitor them by phone and/or email and keep them on the drug. If Grade 2 side effects are reported, we will discontinue drug until the side effect resolves and then resume administration with careful observation for reoccurrence. Monitoring for grades 1 and 2 side effects will include weekly phone calls and/or emails until symptoms subside. If symptoms worsen subjects will visit their primary care physician. Subjects who experience grade 3 symptoms will receive an assessment by our healthcare provider and will be advised to schedule a prompt appointment with their local healthcare provider. We will also encourage them to contact us at any time with questions or concerns.

We will offer an evaluation at NIH for any participant who would like to return for an evaluation of side effects, such as, but not limited to, local participants. This evaluation will include a history and physical examination, discussion with the medical advisory investigator,

and appropriate consults, for example a GI consult.

An informational letter (Appendix F) to the participant's physician will be given to each subject to give to their primary care provider.

8. Subject Safety Monitoring

For CPET, there is literature to suggest that participants with mutations in RYR1 are susceptible to exertional rhabdomyolysis or heatstroke with extreme exercise. We will not be having the patients do extreme exercise, but will take precautions to be ready to prevent these susceptibilities just in case. The precautions include having the CPET performed in a cool room with a fan blowing on the patient, while monitoring the patient's temperature. We will ask the patient if they are experiencing cramping, stiffness, or pain throughout the procedure. If the answer is yes, or if their temperature exceeds 101 degrees Fahrenheit, we will stop the exercise immediately. A STAT blood draw will be done to check serum creatine kinase and potassium levels and urine will be collected for myoglobin. If the patient is medically unwell, the patient will be admitted to the day hospital and an IV will be started immediately for hydration. If the CK, potassium and urine myoglobin levels are elevated suggestive of rhabdomyolysis, they will be treated with sodium bicarbonate and monitored until the rhabdomyolysis is safely resolved.

Investigators will evaluate participants for any foreseeable safety concerns before study procedures are performed. Investigators will allow participants to miss individual procedures (other than the two primary outcome measures— urine collection for 15 F2t-isoprostane levels and 6MWT), at one or all study visits, if safety concerns arise. Examples include: If a participant has a recent history of knee dislocation or knee injury, that participant will be excluded from the Biodex procedure. If a participant has an increased risk of blood clotting, that participant will not perform the arterial occlusion test. If a participant is at NIH and recently recovered from a rhabdomyolysis crisis, that participant will not perform CPET at that visit, but may perform CPET at following visits, if deemed safe at that time. If a participant has certain types of metal in their body, that participant will not perform the MRI procedure at any visit. Investigators will ask participants about potential safety concerns for each procedure and participants are also asked to inform study investigators about potential risk factors in the consent. Investigators will have a participant miss certain non-primary outcome measure procedures, due to safety concerns, at their discretion. Participants will remain enrolled in the study if they are able to safely perform the two primary outcome measures and are also able to safely consume the study medication/placebo, given the expected side effects. Dr. Meilleur can withdraw a participant, at any time, if she believes continuation is not in their best medical interest, or if the participant is unable to comply with the study.

For monitoring for NAC administration, please see previous section under "Steps Taken to Minimize Risk".

For other individual monitoring plans, please see previous section under "Steps Taken to Minimize Risk."

Criteria for stopping procedures in an individual include:

If a patient reports Grade 3 side effects for diarrhea, nausea, vomiting, constipation, or abdominal pain, or any other Grade 3 side effect, according to the NCI Common Terminology

Criteria for Adverse Events v.3.0, we will discontinue their participation.

Criteria for individual subject withdrawal from the study

If Grade 1 side effects are reported, we will monitor them by phone and/or email and keep them on the drug. If Grade 2 side effects are reported, we will discontinue drug until the side effect resolves and then resume administration with careful observation for reoccurrence. Please see Section 7 under "Steps Taken to Minimize Risks" for further details.

9. Outcome Measures

Outcome Measures	Aim 1=Oxidative Stress	Aim 2=Fatigability, Fatigue
Primary	urine 15-F2t-isoprostane	6MWT
Secondary	DCFH assay in muscle biopsy (6 and 12 months only), blood GSH	Timed function tests; Biodex; PROMIS, FACIT, MFI, MRI, MFM, NeuroQoL scales, Myotools
Exploratory	Anaerobic threshold, NIRS; secreted ER calcium monitoring proteins (SERcaMPs) assay in blood and muscle	CPET, EIM, salivary FBI, melatonin, cortisol and DHEA; QoL qualitative interview

Aim 1

Blood and urine samples will be collected **at each visit.** Samples will be analyzed at NIH or shipped to Dr. Milne for urine isoprostane analysis at the Vanderbilt University Ecosinoid Core Facility. De-identified blood samples will also be shipped to Emory University to assay glutathione via mass spectrometry. Needle muscle biopsy will be performed at six and twelve months on up to 20 willing adult participants only.

GSH: Mass spectrometry, fluorescent, and colorimetric assays will be used to measure levels of reduced (GSH) and oxidized (GSSG) glutathione in plasma and whole blood to observe changes in oxidative stress. These assays include deproteinization, derivatization, and/or catalytic reactions.

DCFH: As in the preliminary studies above, DCFH will be used on participant muscle biopsies to analyze intracellular oxidant activity [H2DCFDA (H2-DCF, DCF)].

15-isoprostane-F2: Urine will be assayed for 15-isoprostane-F2, which is formed when arachidonic acid reacts with ROS. A validated GC-MS/MS method will be used to quantify 14-isoprostane-F2.

Anaerobic threshold: Anaerobic threshold and time to anaerobic threshold will be extrapolated from data obtained during CPET.

NIRS: Percentage of local muscle oxygen and oxygen consumption as determined by tissue oxygen index (TOI) and hemoglobin.

SERcaMPs: Measurement of protein level of *Gaussia* luciferase-based secreted ER calcium-monitoring protein (SERCaMP) in blood, primary muscle cells and/or cell lines. (Hendersen et al. 2015)

Aim 2
All procedures for Aim 2 will be performed at the NIH Clinical Center **at each visit.**

CPET: VO2 peak, peak power, test duration of entire test, time to AT, work efficiency (VO2 work rate slope), and ventilator equivalent (VE/VO2) will be obtained.

EIM: An EIM will be used to measure skeletal muscle health. Resistance, reactance and phase of multiple muscles will be measured.

6MWT: Meters walked in 6 minutes will be recorded (to the nearest meter). Distance in meters will be recorded at each minute interval. Speed will be calculated.

Timed tests: Participants will be timed in the performance of the following: 10 meter walk test, supine to stand, ascend 4 stairs, descend 4 stairs. They will be graded on an ordinal scale.

Myotools: Force required for participants to squeeze the grip dynamometer and pinch the pinch gauge. The best effort of 3 trials will be recorded for grip and pinch strength.

Biodex: Biodex will be used to test muscle groups using a dynamic sub-max fatigue test with a repetitive motion. Blood pressure will be taken prior to testing to confirm the patient is not hypertensive (>140/90). Outcome measures will include peak torque measured in newton meters and number of repetitions to 50% decline in peak torque.

Arterial Occlusion Muscle Oxygenation Capacity Test: The tissue saturation index will be calculated as TOI= [O2-Hb] / Tot-Hb. The rate of decline and rise in TOI will be the main muscle tissue saturation measures.

MFM: The final outcome is a percentage based score of each domain and the total score.

PROMIS, FACIT, MFI, NeuroQoL: Questionnaires will be scored based on each scale's requirements.

MRI: The outcomes will be muscle mass via T1, fat composition via T1 to T2 comparison, water composition via T1 to T2 comparison and chemical composition (lipids) via MRS.

Saliva collection: FBI, melatonin, cortisol and DHEA levels will be obtained.

Qualitative interview: Responses to the interview will be obtained and major themes will be identified.

10. Statistical Analysis

a. Analysis of data/ study outcomes

Natural history study (Phase 1 of protocol):

During the natural history phase of the study, we will take one look at the data prior to the final analysis with the following two approaches:

1) After 30 participants complete their baseline visit, we will compare their baseline outcome measure values against healthy controls. For the primary outcomes, 15-F2t-isoprostane and 6MWT distance will be compared against healthy individuals. This analysis will be repeated when all baseline visits are completed.
   Hypothesis*: 15-F2t-isoprostane will be significantly increased in RYR1-RM myopathy patients compared to healthy individuals.*
   Hypothesis: 6MWT distance will be significantly decreased in RYR1-RM patients compared to healthy individuals.
2) After 30 participants complete their 2nd visit at 6 months (prior to starting study drug/placebo), disease progression will be assessed using paired t-tests to determine changes between 0 and 6 month visits for each outcome measure. For the primary outcome measures, 15-F2t-isoprostane and 6MWT will be assessed for change between 0 and 6 months. This analysis will be repeated when all 6 month visits are completed.
   Hypothesis*:* 15-F2t-isoprostane concentration *will not change significantly between 0 and 6 months in RYR1-RM myopathy patients.*
   Hypothesis: 6MWT distance will not change significantly between 0 and 6 months in RYR1-RM patients.

The purpose of the above interim look analyses (under #1) is to confirm that the affected subjects are different from healthy individuals. Because of the rolling nature of the study and the length of time involved to complete the first phase of the study, this allows evaluate whether these outcomes are different from healthy individuals, as expected, in order to confidently proceed with the study. The purpose of the above interim look analysis (under #2) is to characterize the natural history and variability of the RYR1-RM patients for these specific outcome measures and to contribute novel data to the field of RYR1 as soon as possible for future trial design, given that an N of 30 is a large number in rare disease.

Optimal measures for fatigability in RYR1-RM will be determined using paired t-tests for 6MWT distance and speed, time for graded functional tests, and fatigue index for biodex fatigue test. Correlative analysis (Pearson correlation and/or Spearman rho) will be used to examine the linear relationship between subjective fatigue measures (FACIT and MFI) and the salivary fatigue biomarker to the objective fatigability measures mentioned above as well as to compare forced vital capacity to slow vital capacity. Spearman rho correlation, Pearson correlation and or Bland Altman analysis will also be used to examine the relationship between slow vital capacity and forced vital capacity.

Reliability will be assessed for PROMIS, FACIT, MFI, MFM, and NeuroQoL by Cronbach's alpha for internal consistency and by Intraclass correlation coefficient for test-retest. If the test-

retest analysis yields a poor correlation (ICC<0.40), then the scales will not be used in the study since they are not primary outcome measures. However, if they yield moderate to high correlation, we will use the scales throughout the entire study.

Randomized, double-blind, placebo controlled, drug trial (Phase 2 of protocol):
Student's t test will be used to compare treatment to control groups. As mentioned in the Introduction, under "Summary", success of the trial will be defined as finding a significant difference in the primary oxidative stress measure of of urine 15-F2t-isoprostane between the treatment and placebo groups at the termination of the study (significantly decreased urine 15-F2t-isoprostane concentration in treatment group compared to placebo group). A sub group analysis will be performed on adults versus children given the difference in dosing. A paired t test will be used to compare subjects to their baseline values. A student's t test will be used to compare the difference in glutathione between 6 months and 12 months, between the two groups. General linear modeling will be used to control for possible confounders such as gender, age, and baseline values. Log transformed values may be used if there is no bell curve. We will use an alpha of 0.05 to determine significance.

We will use the Grounded Theory Method (GTM) of data analysis to analyze the semi-structured interview data. GTM methodically organizes unique individual experiences collected during interviews into comparable categories so that it becomes possible to find commonalities as well as variations in personal experiences. We use GTM because it is particularly effective for analyzing qualitative interview data and because it is rigorous. A GTM approach is appropriate when there is limited knowledge about a substantive area, such as the fatigue and quality of life of individuals with RYR1-related myopathy.

Select patients will undergo exome sequencing as, occasionally, patients with RYR1-related myopathies with identical *RYR1* mutations have different phenotypes. Exome sequencing may help identify the cause of this difference. Because the RyR1 protein interacts with numerous other proteins, the genes encoding these other proteins may be modifiers of severity. Patients will be selected for exome analysis if they have the same mutations but have a difference of greater than 10% on the MFM32 score. A candidate gene approach will be taken to sequence the genes encoding these other proteins, to evaluate whether they have SNPs or mutations that affect severity. Using the exome, additional mutations in *RYR1* as causative for the phenotypic difference will be ruled out prior to looking at other candidate genes. Genes that encode proteins without a known function in relation the RyR1 protein will not be interrogated so as to avoid unintentional incidental findings.

Healthy volunteer data:

In the first phase of the study, results will be obtained from healthy controls for the biomarkers, NIRS, muscle ultrasound, and MRI. An age-matched analysis will be performed to compare healthy volunteer data to patient data for these outcome measures. We will use an alpha of 0.05 to determine significance. For the comparison to healthy volunteers, age-matched participants will be used. Please see inclusion criteria under healthy volunteers for age grouping.

b. Power analysis
Sample size:

This is a randomized controlled trial with two groups: NAC and Control. For sample size planning purposes, we have used glutathione data, pre and post NAC treatment, from an HIV population (Muller et al. 2000). Assuming the NAC treatment group will have a delta (post-treatment glutathione – pre-treatment glutathione) of 1.7 and delta standard deviation of 2.34, and the control group will have a delta of 0 and delta standard deviation of 2.80, for a 2-sided alpha of 0.05 and 80% power we will need 38 patients per group (2-group Satterthwaite t-test of equal means and unequal variances).
We need 76 participants, per group, based on the current sample size. We expect 24 drop outs, per group. Therefore, our accrual ceiling for all participants is 200.

The primary analysis will be an intent-to-treat (ITT) analysis, i.e. all randomized participants will be included in the analysis. The mechanism for missing data will be evaluated. If missing data are unrelated to the intervention or primary outcome (i.e. missing completely at random or missing at random), the analysis will be based on multiple imputations (20-40 imputed datasets, depending on the extent of the missing data) with a pooled average of the 20-40 imputations included in the final dataset and used in hypothesis-testing analyses. Minimum and maximum value constraints for imputed data will be determined using *per protocol* averages.

Because of uncertainties regarding the true effect size of NAC treatment in our patient population, and the rare nature of this disease possibly limiting accrual, we plan to perform a sample size reevaluation and interim analysis once 30 patients have completed the trial, i.e., when 40% of our potentially achievable accrual reach Month 12.

For the sample size reevaluation, and based on the observed data so far, we will calculate the following: the control group delta and its standard deviation and the standard deviation of the delta for the active arm. Based on these observed parameter values for urine 15-F2t-isoprostane, we will re-calculate the sample size and compare it to the current N=76.

Based on O'Brien-Fleming boundary calculations (O'Brien and Fleming, 1979), the interim analysis will result in one of 3 actions:

1. If there is strong indication of no benefit of NAC in decreasing 15 F2t-isoprostane levels, i.e. if the observed standardized treatment effect is less than -3.36, we will consider stopping the study early for futility.
2. If there is no clear indication of benefit of NAC decreasing 15 F2t-isoprostane levels, i.e. if the observed standardized effect size is between -3.36 and 3.36, we will continue the study and analyze the data at the completion of the study.
3. If there is strong indication that the NAC treatment decreases 15 F2t-isoprostane levels, i.e. if the observed standardized treatment effect is greater than 3.36, we will consider stopping the study early for efficacy.

Stopping the trial early when there is strong evidence for futility or efficacy will reduce patient burden, decrease excess costs, and provide a potential treatment sooner for this rare disease, which currently has no treatment.

The primary outcome measure is being changed, initially due to pipette calibration error in the lab at NIH. Additional review of our data at Emory after sending a second batch of samples and

further literature review identified further justification for changing the primary oxidative stress biomarker from glutathione (GSH) to 15-Ft2-isoprostane:

--The processing of GSH and its oxidized form GSSG often results in methodological artifacts. For instance, sample acidification for protein precipitation leads to an increase in GSSG levels thus decreasing GSH:GSSG ratio (Marrocco et al. 2017). The assay is sensitive to exposure to air/time and sample volume and is therefore prone to error. The aforementioned methodological artifacts are not unique to this protocol and have been described previously (McGill et al. 2015).

--During the current protocol, such methodological issues arose at Emory in addition to the pipette calibration issue at NIH. For example, when the second sample for each patient was rerun at Emory, including samples unaffected by the pipette error, the results between the first patient and second patient sample were unreliable. These methodological errors in GSH as a biomarker and the recurrent, significant time delays for processing by Emory, in addition to the pipette error, prompted a change in primary outcome measure from GSH to 15-Ft2-isoprostane.

--15-Ft2-isoprostane was collected on each individual at each visit as a secondary outcome measure for oxidative stress throughout the life of the protocol. The change from GSH to 15-Ft2-isoprostane is being made prior to treatment allocation un-blinding and prior to statistical analyses, which is the optimal time to change a primary endpoint when such a change is required (Evans, 2007). Changes in primary endpoints are reported in approximately 30% of clinical trials (Ramagopalan, 2014).

--Elevated oxidative stress is a hallmark pathological feature in RYR1-RM and has been identified in several pre-clinical models of the disease (Durham et al. 2008, Dowling et al. 2012, Lee et al. 2017, Michelucci et al., 2017). In particular, lipid peroxidation of mitochondrial membranes has been implicated as a central pathomechanism that leads to severe mitochondrial dysfunction and/or death in the RYR1Y522S/W mouse model (Durham et al. 2008). Notably, treatment of the aforementioned mice with N-acetylcysteine (NAC), (ad lib via drinking water for 8 weeks) rescued lipid peroxidation and concomitantly prevented previously observed decrements in force generation (Durham et al., 2008). These findings have recently been supported by a longer-term study of NAC treatment in the same mouse model in which NAC treatment led to a decreased number of structural cores and improved skeletal muscle function (Michelucci et al., 2017).

--15-F2t-isoprostane has been reported as the most reliable in vivo biomarker of oxidative stress and is a measure of lipid peroxidation (Milne et al. 2007, van 't Erve et al., 2017). Moreover, a typical 15-F2t-isoprostane concentration has been established through substantial data collection from otherwise healthy volunteers (n= 1881) allowing comparisons to be made to disease populations (van 't Erve et al., 2017). Finally, when our participants' baseline and 6 month (untreated) isoprostane levels were compared, the findings were not significantly different, suggesting reliability of this biomarker in our population, in contrast to our GSH data analysis of the same time points.

--In a recent crossover trial, conducted in 36 individuals with low resting GSH concentrations, NAC supplementation (2 × 600 mg, twice daily, for 30 days) led to a 22% reduction in 15-F2t-isoprostane concentration and improved exercise performance (Paschalis et al. 2017). These findings are pertinent for this protocol as it has been demonstrated pre-clinically that RYR1-RM models have low resting GSH concentrations, and both 15-F2t-isoprostane concentration and improved exercise performance are being tested in our trial. Additional antioxidant clinical trials have also shown a beneficial effect of treatment on 15-F2t-isoprostane concentration. In particular, an α-tocopherol (vitamin E) antioxidant trial in smokers (n= 81 placebo; n= 82 vitamin E) demonstrated that consuming 400 IU vitamin E daily resulted in a statistically significant (21%) difference in corrected urinary F2-isoprostane concentration compared to placebo following 32-months treatment (Guertin et al. 2016).

In combination, these findings strengthen the rationale for using 15-F2t-isoprostane concentration as the primary outcome measure for this protocol in place of GSH. The sample size recalculation for 15-NR-0072 was therefore performed using 15-F2t-isoprostane data from our study. Dr. Paul Wakim, Clinical Center Statistician, was unblinded for the sample size recalculation. All investigators remain blinded until the study is complete. Dr. Wakim used the current study data on isoprostane levels from participants in the treatment vs. placebo groups to calculate the N required to complete the study. The N achieved was 182, which is not feasible in this rare disease population and the study is being closed to data analysis.

11. Human Subjects Protection

a. Subject selection

Subject selection will be equitable as much as possible due to the rarity of RYR1-related myopathy. All ages within the specified range, races, and both genders will be accepted. Individuals with a genetic mutation in *RYR1* will be prioritized to decrease the rate of screening failures. Individuals with a muscle biopsy showing central cores will be prioritized to decrease the variability in the sample.
Subject selection for healthy volunteers will be equitable.

b. Justification for inclusion of children

Children age 7 years old and older are affected with this disease and are affected with fatigue. This drug in this route of administration has a good safety profile in children at this dose for other indications. Children <7 years of age will be excluded as they will not be able to follow the commands in order to perform all of the outcome measures.
Healthy volunteer children are needed to provide age-matched groups as controls for biomarkers, NIRS, muscle ultrasound, and MRI.

c. Justification for exclusion of other vulnerable subjects

Those unable to provide consent due to cognitive issues will be excluded, due to the requirement to be able to respond to questionnaires such as PROMIS and FACIT.

d. Justification of sensitive procedures (use of placebo)
The use of a placebo is necessary to determine the effects of NAC on this population. Without the placebo, we would not be able to determine if NAC has a significant effect on participants with RYR1-RM. Use of a placebo will allow us to draw stronger conclusions about the effects of NAC than if we conducted the study without a placebo.

e. Safeguards for vulnerable populations (e.g. DPA, pregnancy testing, contraception use, ethics consult, HSPU involvement)
Safeguards: Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation (Appendix L)" prior to obtaining consent, 3) assuring that there will be no direct solicitation of employees or staff, and, 4) Independent consent monitoring will be provided by the NIH HSPU.

This study collects sensitive information on pregnancy status, which will be in the participant's NIH medical record. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures. All research activities will be conducted in as private a setting as possible, limiting personnel present.

For pediatric patients, we will have parental consent and minor assent. Pediatric staff and pediatric equipment will be used. During blood draws, a local anesthetic cream such as Eutectic Mixture of Local Anesthetics (EMLA) may be used to minimize the pain for young children. For pediatric participants, no more than 5 mL/kg will be drawn in a single day, and no more than 9.5 mL/kg will be drawn over any eight-week period.

Blood will not be drawn on participants if they are known to be anemic (Hgb < 8 gm/dl) per medical history, or if their medical condition causes an investigator to believe that blood drawing might be detrimental to the participant.

Parents can accompany their child to the MRI suite and watch their child through a window during the procedure. During the procedures, there will be music and/or video to distract the child. All participants will be able to communicate with the MRI staff at all times.

For women of childbearing age, we will do pregnancy testing to exclude women who are pregnant at the beginning of the study. We will also exclude women who are breastfeeding. Women who are sexually active will require contraception. Acceptable contraception includes barrier methods (contraceptive sponge, diaphragm, female and male condoms), hormonal methods (oral contraceptives, the patch, shot/injection, and vaginal ring), implantable devices (implantable rods and intrauterine devices), and permanent birth control methods (sterilization implant and surgical sterilization).

f. Qualifications of investigators

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training.

Suzanne Wingate, PhD, ANP-BC is a board certified adult nurse practitioner and the Clinical Director of NINR. When the study was changed to Data Analysis only, she began to provide oversight for the study, but there is no clinical or medical management required for subjects in this phase.

Katherine Meilleur, Ph.D., PNP-BC is an assistant clinical investigator in the Tissue Injury Branch/NINR. She is a board certified pediatric nurse practitioner involved in study design, data analysis, interpretation of results and all procedures. She is an investigator authorized to obtain informed consent from participants.

James Dowling, MD, PhD is an assistant professor at the University of Toronto and is a Medical Doctor, licensed and board certified pediatric neurologist. He is involved in study design, data analysis, interpretation of data, and is an investigator authorized to obtain informed consent.

Carsten G. Bonnemann, M.D. is the Chief of the Neuromuscular and Neurogenetic Disorders of Childhood Section of the NGB/NINDS. He is a Medical Doctor and a licensed and board certified pediatric neurologist. He is involved in study design, data analysis, interpretation of data, and is an investigator authorized to obtain informed consent from participants.

Irene Chrismer, BSN, RN has been a research nurse in the NINR for 1 year. Prior to that she worked at the Children's National Medical Center cardiac unit as a staff nurse. She is a board certified nurse and will be involved in eligibility screening and patient care. Due to her experience over that past year and dedicated involvement on this protocol, she is an investigator authorized to obtain informed consent from participants.

Diana Bharucha, MD, is a pediatric neurologist and clinical research fellow for NGB/NINDS. She has several years of experience with RYR1 patients and will be involved with data collection. She is authorized to obtain informed consent from participants.

Reghan Foley, MD, is a clinical Research Fellow for NGB/NINDS. She has previous experience at the Dubowitz Neuromuscular Centre, Institute of Child Health at the University College of London. She will be involved in patient referral, study design, data analysis, and interpretation of results. She is an investigator authorized to obtain consent from participants.

Monal Punjabi is a PharmD student, Class 2015, at Rosalind Franklin University of Medicine and Science. She is a summer student and special volunteer. She will assist with the IND application and assist as need. She is not authorized to obtain informed consent.

Minal Jain, PT, DSc, PCS, Melissa Waite, PT, and Bart Drinkard, PT are licensed physical therapists in the Rehabilitation Medicine Department of the Clinical Center. They will administer outcome measures (fatigue and fatigability). They are not authorized to obtain informed consent.

Tanya Lehky, MD, is a physician at the Clinical Center. She will perform the EIM procedure. She is not authorized to obtain informed consent.

Kimberly Amburgey, MS, CGC, is a board certified genetic counselor at the University of Toronto. She is involved in the recruitment of patients and study design. She is an investigator authorized to obtain informed consent.

Ami Mankodi, MD, and Christopher Grunseich, MD, are licensed and board certified neurologists. They will be involved in patient evaluation and muscle biopsy procedures. They are investigators authorized to obtain informed consent from participants.

Sonia Razaqyar, BA, is a postbac at the NINR. She earned a BA with honors in biochemistry. She will be involved with data entry, data analysis, and patient coordination. She is an investigator not authorized to obtain informed consent from participants.

Monique Shelton, BA, is a postbac at the NINR. She earned a BA in biology and Spanish. She will be involved with data entry, data analysis, and patient coordination. She is an investigator not authorized to obtain informed consent from participants.

Anna Kuo, BA, is a postbac at NINR. She earned a BA in neuroscience and behavior. She will be involved with data entry, data analysis, and patient coordination. She is an investigator not authorized to obtain informed consent from participants.

Joshua Todd, PhD, is a postdoc at NINR. He earned a PhD in biomedical sciences and a BS in sports science. He will be involved in data collection, data entry, data analysis, and dissemination of results. He is an investigator authorized to obtain informed consent.

Allen, Carolyn, DNP, MS, CRNP-F, is a Family Nurse Practitioner at NINR. She will be involved in data collection, data entry, data analysis, interpretation of results and procedures, and dissemination of results. She is an investigator authorized to obtain informed consent.

Jessica Witherspoon, PhD, DPT, is a postdoc at the NINR. She will be involved in data collection, data entry, data analysis, and dissemination of results. She is an investigator authorized to obtain informed consent.

Etsuko Tsuchiya, BS, is a research coordinator at U. Toronto and will coordinate samples and data between the 2 sites. She is an investigator not authorized to obtain informed consent.

Darren Michael, PhD, is a scientist who has developed a fatigue biomarker in saliva and works at Hyperion Biotechnology Inc. He will oversee and participate in the processing and analysis of samples, as well as the interpretation and publication of the results. He is an investigator not authorized to obtain informed consent.

12. Anticipated Benefit

This study may offer a temporary direct benefit to adult and pediatric participants in the study drug group, such as reducing oxidative stress, fatigue, and fatigability. Reduced

oxidative stress decreases cellular necrosis and improves energy efficiency. In turn, this would allow the participants to improve their energy level. (Hybertson et al., 2011) Additionally, improved energy efficiency translates to reducing fatigability, thereby improving endurance, which was noted by Dowling et al., in the RYR1 zebrafish model with NAC administration.
There will be no benefit for healthy volunteers.

13. Classification of Risk (for the study as a whole)

a. For adults: More than minimal risk

b. For Adults without consent capacity (not applicable)

c. For Children (Patients): 45CFR46.405 Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual child
For Children (Healthy Volunteers): 45CFR46.406 Research involving no greater than a minor increment over minimal risk and no prospect of direct benefit but likely to yield generalizable knowledge

d. Overall risk and benefit consideration
The risks are reasonable in relation to anticipated direct benefit.

14. Consent Documents and Process

a. Designation of those obtaining consent
Study investigators, designated as able to obtain consent in section 11f above, will obtain informed consent. All NIH study investigators obtaining informed consent have completed the NIMH HSPU "Elements of Successful Informed Consent" training. Assent will be obtained from minor subjects.

b. Consent procedures
All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. Written assent will be obtained, when possible, from minors age 7 and older. Minors who reach age 18 while enrolled will be consented as adults for continued participation.

If eligible, the subjects will undergo an in-person written consent at NIH. During the consent process, the child and custodial parent will sign the consent form. The original consent form will be filed in the participant's NIH chart. A copy of the completed signed consent will be returned to the participant. The consenting investigator will document the consent procedure in the NIH medical record.

For pediatric patients, if the parents are married, written consent may be obtained from one parent only. For pediatric healthy volunteers, consent from both parents is required, even if parents are married. If the parents are not married, written consent will be obtained from: 1) the

custodial parent if only one parent has legal custody, or 2) from both parents if they share legal custody for medical decision-making. For unmarried parents, signature of one parent will suffice if the other parent is deceased, unknown, incompetent, or not reasonably available. When signature of both parents is required, written consent will be obtained in-person from at least one parent. When the second parent is unable to attend the consent process conference in person, the following telephone process to obtain written consent will be used.

Telephone Consent Procedures: The unavailable parent will be provided with a copy of the consent form, usually by fax, email, or hard copy mail. Once the consent form is received, an Investigator authorized to obtain consent will arrange for a telephone call with the parent in the presence of a witness to review study and the consent form and to answer any questions. If the parent cannot arrange for a witness in his/her location, the consenting investigator will have a witness present for the teleconference at NIH. Once the parent agrees to his/her child's participation, the parent and witness (if present with the parent) will sign and date their copy of the consent form. The Investigator will enter a note documenting the consent process in the Medical Record. The 2nd parent will return their signed copy to the Investigator. Once the copy with the parent signature is received, the Investigator and witness (if present at NIH) will sign and date the 2nd parent consent form, place the original copy with all signatures in the Medical Record, retain a copy for research records, and mail a copy to the parent.

The telephone consent process described above may be used to obtain written re-consent from participants who are already enrolled. Written informed consent will be obtained from minor participants when they reach the age of consent for continued use of their samples or data. The telephone consent procedure detailed above may be used for such consent.

For NIH employees: Consent may be obtained by a co-worker. If so, independent consent monitoring will be provided by the NIH HSPU.

c. Consent documents

The consent contains all required elements. The consents attached to the protocol include adult patient and healthy volunteer populations (or parent of minor participants) and two different minor assents (ages 7-13 and 14-17), both for patients and healthy volunteers.

15. Data and Safety Monitoring

An independent monitoring committee will be put into place for Phase 2 of the study, including three experts in the field of neurology.

This committee will meet once every 3 months after the start of Phase 2 and is comprised of:

Andrew Mammen, MD, PhD, NIAMS, Muscle Disease Unit Leader, specializes in patients with myositis and autoimmune toxic myopathies and muscular dystrophies.

Ronald Cohn, MD, University of Toronto, is the Chief of Clinical and Metabolic Genetics at the Hospital for Sick Children and specializes in neuromuscular diseases.

Joan Austin, PhD, University of Indiana, Professor of Nursing at U. Indiana and specializes in pediatric epilepsy and chronic illnesses in children. She has served on Data and Safety Monitoring Boards for 2 national trials and a number in Indiana.

a. Data and safety monitor
This study will be monitored by the PI for Phase 1 of the study and by an independent monitoring committee for Phase 2.

b. Data and safety monitoring plan
Parameters to be monitored include liver function tests at the baseline visit and gastrointestinal symptoms throughout the 2nd phase of the study.

Liver Function Testing (LFT) in patients with RYR1-RM: LFTs are often elevated in our patient population. In patients with neuromuscular disease, the following criteria must be met, in order to determine impaired liver function. These guidelines will be used for determining impaired liver function in patients with RYR1-RM.
Evidence of moderate to severe hepatic involvement in this patient population is defined as:

> ALT ≥ 8x upper limit of normal (ULN) with total bilirubin 2x ULN (plus >35% 'direct' bilirubin) and/or INR >1.5
> or
> GGT > 2-3x ULN with bilirubin 2x ULN (plus >35% 'direct' bilirubin) and/or INR >1.5

An increase to >3 times above the upper limit of normal in ALT, AST, ALP and/or GGT, not observed pre-drug/placebo, will be reported. A change in the transaminases (ALT, AST, ALP or GGT) from normal elevation for our population per its natural history, which is < 3X ULN, plus any increase in INR>1.5 or total bilirubin>2X ULN will trigger the action of immediate consultation with the MAI, Dr. Bonnemann regarding a decision of withdrawal of the patient. If Dr. Bonnemann is unavailable, Dr. Traynor, Dr. Grunseich, Dr. Mankodi or Dr. Fischbeck will be consulted. If medical consultation results in the decision to withdraw, the patient will be withdrawn immediately.

c. Criteria for stopping the study or suspending enrollment or procedures

For the first drug-related SAE, the study will be stopped in that subject. The SAE will be reported immediately (as soon as the PI becomes aware) to the IMC and IRB, and the PI will follow their instructions on what research activities can proceed or need to be stopped. For the second same related SAE, the study will be temporarily stopped, including research activity with enrolled participants, until IRB review, unless the IRB specifically permits certain research activity to continue.

The IMC, in consultation with the IRB, will review the data, unblinded if necessary, and will recommend whether to restart the intervention.

16. Quality Assurance

16.1 Quality assurance monitor

As per the "National Institute of Nursing Research Intramural Research Program Policy on Data and Safety Monitoring (3/1/12)" Section IV D #5, the NINR Clinical Director will designate trained staff to monitor the progress of a clinical protocol.

16.2 Quality assurance plan

All study staff will maintain current training in good clinical practice and protection of human subjects, and will complete other protocol- and role-specific training, as needed.
This Minor Increase over Minimal Risk Study will follow guidelines noted in the "National Institute of Nursing Research Intramural Research Program Policy on Data and Safety Monitoring (3/1/12)" Section IV H #1 and be monitored at the following intervals: within 3-6 months of first subject enrollment, at least annual interim monitoring, and as needed.

17. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD.

An electronic spreadsheet (Appendix K) is saved on the investigators' secure shared drive, which tracks and records when each AE occurred, when the PI became aware of the event, whether the event is unexpected (with regard to information in the protocol, consent forms, and literature), whether the event is at least possibly related to the research, whether the event increased the risk to participants beyond that previously known or undermined the integrity of study data, how the event was managed, whether/when and how the event resolved, and required reporting for the event. Each AE will be reviewed with the IMM, Bryan Traynor, MD. Bryan J. Traynor, MD, PhD, is a tenured investigator in the Neurogenetics Branch of the National Institute of Aging (NIA) and is an adult neurologist. He is currently the chief of the Neuromuscular Diseases Research Section of the NIA. Dr. Traynor serves as the Independent Medical Monitor (IMM) for this protocol and will review each AE with the PI. He is an investigator not authorized to obtain informed consent. Further investigation of AEs will be directed to the MAI, as needed. AEs will be reported to the IMC, regardless of PI classification. All adverse events will also be submitted to and reviewed immediately by the NINR Clinical Director. All events will be submitted to the IMC for review. Communication to and from the medical monitors will be in writing.

Serious unanticipated problems, serious adverse events (including deaths) that are not unanticipated problems, and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event, unless immediate reporting is waived for specific serious adverse events as noted below. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

To avoid deviation reports for anticipated missed visits/procedures

It is anticipated that participants in this study will occasionally miss or fail to complete an assessment or

procedure, due to issues such as discomfort, a medical contraindication, or failure to complete a procedure or visit within protocol-specified time frames. Omissions such as these of non-primary outcome measures will be considered expected events and not protocol deviations provided they are infrequent and do not include data needed to assess safety or the primary study outcome. Cumulative proportions of these missed events in the study population will be presented to the IRB annually. In addition, the rate of omissions will be monitored by the Investigators. If an individual misses more than 15% of the required assessments/procedures or if more than 15% of the participants miss completion of the same assessment or procedure, it will be considered a deviation and a deviation report will be sent to the IRB within two weeks.

If the total number of expected items (study visits, study assessments/procedures) is less than 16, then two or more missed items are reportable. If the total number of expected items is greater than 16, then, if more than 15% are missed, it is reportable.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

The PI will immediately report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The PI will record nonserious AEs and report them to the Sponsor within 14 days.

18. Alternatives to Participation

Because this study is a clinical trial to determine the effects of NAC on patients with RYR1-related myopathy, it is necessary for subjects in the trial to take NAC or placebo. Patients may not take NAC prescribed by their own physician during the study. For patients who do not wish to take NAC or placebo or who wish to take it by their own physician, the alternative is to not participate in the trial. There are no therapeutic alternatives.
For healthy volunteers, the alternative is to not participate.

19. Privacy

All research activities will be conducted in as private a setting as possible, limiting personnel present.

20. Confidentiality

a. For research data and investigator medical records: see below for research data. Medical records will be stored in CRIS and CTDB behind the NIH firewall. For photographs and videos, when possible, the patient's face and other identifying marks will not be included in the recording or photograph in order to protect confidentiality. Electronic records, videotapes, photographs, and other data, such as MRI scan data, will be stored on encrypted, password protected servers only accessible by authorized NIH associate investigators. Coded medical history will be stored in CTSS. PROMIS and NeuroQol data will be entered directly by participants into Assessment Center Lite, which is also behind the NIH firewall. Samples sent to other universities, such as U. Toronto and Emory, will be de-identified. Samples sent to other universities for fee-for-service analyses, such as Vanderbilt and Emory Universities, will be de-identified. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

b. For stored samples: Stored samples will be kept behind a double lock or will be coded and kept behind a single lock.

c. Special precautions
Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

For hard copy data/records: Hard copies of questionnaires will be kept in locked file cabinets in locked offices at NINR as well as a participant identification key. All participant data will be coded for the purpose of data analysis and sharing between listed investigators.

For samples without identifiers (coded or unlinked): single lock will be used.

For electronic data with identifiers or recognizable facial images: password protected files on secured servers will be used. Quantitative data in the form of results of rehabilitation medicine testing and responses to questionnaires will be stored in excel, SPSS, and/or Mplus files on encrypted computers at the same sites. Qualitative data (digital recordings) in the form of responses to open ended questions will be collected and retained on encrypted computers at NIH on Nvivo software (offices of Dr. Meilleur's team, NINR). Recordings of qualitative data will be destroyed once the study is completed. Data will be submitted to an online database behind the NIH firewall (CTDB).

Only investigators listed on the protocol will have access to records, data, and samples. If sponsors, monitors or auditors outside of study investigators will need access, they will have access to coded data. Results will be posted on cctrials.gov

21. Conflict of Interest

a. Distribution of NIH Guidelines
NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest
There are no conflicts-of-interest to report for NIH investigators. Non-NIH investigators will abide by the conflict-of-interest policies of their own institutions. We have consulted with DEC and there are no conflicts of interest for Dr. Michael or Hyperion.

c. Role of a commercial company or sponsor
BioAdvantex Pharma Inc will provide complementary drug (PharmaNAC) and placebo oral effervescent pills to NIH for the RTC. Under a non-disclosure agreement, the company will also disclose information regarding description and composition of the drug product and placebo, certificate of analysis and the master production document certificate that contains information about manufacturing, packaging and quality assurance for both NAC and placebo. BioAdvantex has granted permission to cross reference drug master file for NAC and environmental analysis requirement certification. NIH and University of Toronto will provide the protocol and the results of the study to BioAdvantex. Personal identifiers of participants will not be shared with the sponsor.

22. Technology Transfer

An MTA (MTA-15-002) is in place with Dr. Michael at Hyperion Biotechnology Inc., which states that deidentified data will be owned by NINR, but the salivary fatigue biomarker will be processed at Hyperion. MTAs have been obtained to transfer de-identified muscle tissue samples and de-identified genetic mutations to Dr. Andy Marks at Columbia University.

A CTA (NR-CTA-15-001) has been started with BioAdvantex Pharma. The CTA states that BioAdvantex Pharma will provide the drug, but the NINR is responsible for sponsoring the study, and Dr. Meilleur is the sponsor of the IND. An MTA is not required for sending to samples on a fee-for-service basis to Vanderbilt and Emory Universities.

23. Research and Travel Compensation

Patients:

Lodging**,** meals and travel will be provided to patients only by the NINR. In the case of minors or adults who have difficulty traveling alone due to their myopathy, NINR will also pay for travel and lodging for one caregiver. In the case of minors, the caregiver will be the legal guardian. There will be no other payment or reimbursement. Meal vouchers will be provided to the adult patient or the parent of a minor (for the child's meals).

For patients who are staying in a hotel off campus, complementary shuttles are available between their hotel and NIH. If patients are at NIH after these routine shuttles have stopped for the day, taxis will be provided for them, and covered under the protocol. NINR staff will arrange the taxi. Research study staff will be present after late-day procedures, if needed, to assure transportation needs are addressed.

NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

Healthy Volunteers:

Travel and meals will be provided to volunteers by the NINR. In the case of minors, NINR will also pay for travel and meals for one legal guardian. Only healthy volunteers will be paid for their participation. Each volunteer will be provided with $50 compensation. For those undergoing an MRI, an additional $25 will be provided. Only 2-8 people from each age group will be asked to undergo an MRI. If the participant must stay the night in order to participate, one night of lodging will be provided to the participant and their legal guardian (in the case of minors).

For volunteers who are staying in a hotel off campus, complementary shuttles are available between their hotel and NIH. If volunteers are at NIH after these routine shuttles have stopped for the day, taxis will be provided for them, and covered under the protocol. NINR staff will arrange the taxi. Research study staff will be present after late-day procedures, if needed, to assure transportation needs are addressed.

NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

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25. Attachments/ Appendices

A. Visits and Procedures Schedule
B. Recruitment materials (combined patient wording, patient flyer, HV flyer)
~~C. NIH recruitment advertisement~~
D. Recruitment letter
E. Eligibility checklist
F. Clinician information letter
G. Pre-screen Questionnaire
~~H. Healthy volunteer recruitment flyer~~
I. Needle Aspiration Muscle Biopsy Procedure Information Sheet
J. Medication/Placebo Information and Instructions Sheet
K. Adverse Event Spreadsheet
L. NIH Information Sheet on Staff Research Participation
M. Cover Letter for Continued Use for Minors who turn 18

26. Consent Forms

Adult subject consent
Child Subject assents (ages 7-13 and 14-17)
Healthy Volunteer: Adult Consent
Healthy Volunteer: Child assents (ages 7-13 and 14-17)
Consent for Continued Use for Minors who turn 18