Randomized, Placebo-Controlled Parallel Group Clinical Trial of Nicotinamide Riboside to evaluate NAD+ Levels in Individuals with Persistent Cognitive and Physical Symptoms After COVID19 Illness ("Long-COVID")

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STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and the applicable regulatory requirements, United States Code of Federal Regulations (CFR) Title 45 CFR Part 46 and Title 21 CFR Parts 50, 56, and 312.

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
AE	Adverse Event/Adverse Experience
ASL	Arterial Spin Labeling
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BOLD	Blood Oxygen Level Dependent
BRIEF-A	Behavioral Rating Inventory of Executive Function – Adult Version
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CRF	Case Report Form
CNS	Central Nervous System
CNY	Charlestown Navy Yard
Co-I	Co-Investigator
COVID19	Coronavirus SARS-CoV-2
CPT	Continuous Performance Test
CSF	Cerebrospinal Fluid
DKEFS	Delis-Kaplan Executive Function System
DNA	Deoxyribonucleic acid
DOB	Date of Birth
eCOG	Everyday Cognition Scale
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FABP	Fatty Acid Binding Protein
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFAP	Glial Fibrillary Acidic Protein
GRAS	Generally Recognized as Safe
HC	Healthy Control
HIPAA	Health Insurance Portability and Accountability Act
HRC	Human Research Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IPAQ	International Physical Activity Questionnaire
IRB	Institutional Review Board
MCI	Mild Cognitive Impairment
MDU	Memory Disorders Unit
MGH	Massachusetts General Hospital
MRI	Magnetic Resonance Imaging
MRN	Medical Record Number
NAB	Neuropsychological Assessment Battery
N	Number (typically refers to subjects)
NAD^+	Nicotinamide Adenine Dinucleotide
NFL	Neurofilament light-chain protein
NMN	Nicotinamide mononucleotide

NR	Nicotinamide riboside
PBO	Placebo
pCASL	Pulsed Continuous Arterial Spin Labeling
PCL-C	Post Traumatic Stress Disorder Checklist – Civilian
PHRC	Partners Human Research Committee
PI	Principal Investigator
PT/INR	Prothrombin Time and International Normalized Ratio
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
rsBOLD	Resting State Blood Oxygen Level Dependent
rsfMRI	Resting State Functional Magnetic Resonance Imaging
SAE	Serious Adverse Event
SCD	Subjective Cognitive Decline
TMT	Trail-Making Test
TOPF	Test of Premorbid Functioning
TSH	Thyroid Stimulating Hormone
UCH-L1	Ubiquitin Carboxy-Hydrolase-L1
WAIS-III	Wechsler Adult Intelligence Scale – 4th edition

1. ETHICS/PROTECTION OF HUMAN SUBJECTS

1.1 Institutional Review Board (IRB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21 Part 56 of the United States of America Code of Federal Regulations (CFR) relating to IRBs.

1.2 Ethical Conduct of Study

The study will be conducted in accordance with GCP defined by the International Conference on Harmonisation (ICH) and the ethical principles of the Declaration of Helsinki.

1.3 Subject Information and Consent

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations, and ICH Guidance Documents pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, subjects will be informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. Subjects will be given adequate time to ask questions and become familiar with the study prior to providing consent to participate. Subjects will give their written consent to participate in the study and will be provided with a copy of the fully executed consent form for their records.

If the subject is not fully competent and able to provide consent, he or she will not be eligible to participate in this study. Our study population of scientific interest are individuals with persistent symptoms related to the novel coronavirus SARS-CoV-2 (COVID19); subjects in this category will be expected to have full capacity to consent unless due to another exclusionary factor.

2. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 SARS-CoV-2 infection

There have been over 22 million cases and 350,000 deaths from COVID19 infection in the USA to date, and >5-fold these numbers worldwide[1]. COVID19 infects people of all ages and backgrounds, but it is more common and severe in older adults, people with pre-existing conditions, and racial and ethnic minorities[2,3]. COVID19 is a multi-system disease, especially affecting immune, respiratory, cardiovascular, gastrointestinal, musculoskeletal and nervous systems[4-6]. Initially thought to be an acute illness like the flu, resulting in death for some or full recovery in most, we are now appreciating that substantial numbers of COVID19 illness survivors experience persistent, significant and sometimes disabling symptoms. Variously called "Long-COVID," "COVID long-haulers," or "chronic COVID syndrome", some of the most prominent symptoms are neurologic and neuropsychiatric, including cognitive impairment ('brain fog'), headache, neuropathy and paresthesias, muscle aches and weakness, as well as systemic symptoms of fatigue, shortness of breath, hair loss and pain[5,7-9]. As a new clinical entity, little is yet known about the nature of Long-COVID and virtually nothing about its management.

2.1.2 Nicotinamide Riboside Rationale

Nicotinamide riboside (NR) is a form of vitamin B3, naturally found in low levels in milk, that is a precursor to nicotinamide adenine dinucleotide (NAD+). NAD+ is a central metabolic cofactor that functions as the primary electron donor in the mitochondrial respiratory chain, regulates the activity of various metabolic pathway enzymes in glycolysis, Kreb's cycle, and fatty acid oxidation and enables signaling for major cellular metabolism processes involving sirtuins (Figure 1)[10,11]. These processes are critical for maintenance of synaptic plasticity and protection against neurodegeneration. Neuronal mitochondrial dysfunction occurs with advancing age, but this is especially pronounced in pathological brain aging, such as with AD or vascular cognitive impairment. NR is a safe, well-tolerated, potent NAD+ precursor available as a "generally recognized as safe" (GRAS) dietary supplement[12]. It has promising pharmacokinetic and systemic pharmacodynamics properties and has been shown to ameliorate bioenergetic, behavioral and pathological abnormalities in mouse models of neurodegeneration[13-15].

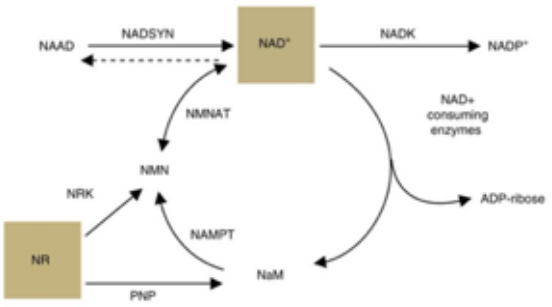

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Figure 1. Nicotinamide riboside (NR) enters the NAD^+ salvage pathway through conversion to nicotinamide mononucleotide (NMN) via nicotinamide riboside kinase (NRK) or conversion to nicotinamide (NaM) via purine nucleoside phosphorylase (PNP). From there, NMN (or NaM after modification by NAMPT) is converted into active NAD^+ and shunted into a variety of metabolic processes. NAD^+ consuming enzymes, such as sirtuins, drain the NAD^+ pool, which must be replenished. Declining activity of nicotinamide phosphoribosyltransferase (NAMPT) with age is implicated in declining levels of NAD^+ with age. NR supplementation bypasses this rate-limiting step by introducing NAD^+ precursors directly into the salvage pathway[16].

2.1.3 Nicotinamide Riboside and SARS-CoV-2

SARS-CoV-2 is predicted to preferentially localize to mitochondria and it has recently been shown to disrupt the NAD metabolome[17]. It has been suggested that SARS-CoV-2 dysregulates NAD gene set. Particularly, it has been shown to down-regulate the synthesis of NAD from tryptophan and nicotinic acid (NA), and to up-regulate the synthesis capacity from NR[18]. SARS-CoV-2 is also known to induce a cytokine storm that increases the circulation of inflammatory cytokines (e.g. Interleukin 1, tumor necrosis factor alpha)[19,20]. As such, it has been hypothesized that NR may be an effective intervention for COVID-19 by increasing NAD+ levels and reducing inflammation[18]. Most recently, a randomized, open-label, placebo-controlled, phase 2 trial examined the effect of a combination of L- serine, N-acetyl-L-cysteine (NAC), NR, and L-carnitine tartrate in the recovery of individuals with COVID-19, over a 14-day period[21]. Findings showed that the combination of these metabolic cofactors significantly reduced time of recovery (Figure 2)[21].

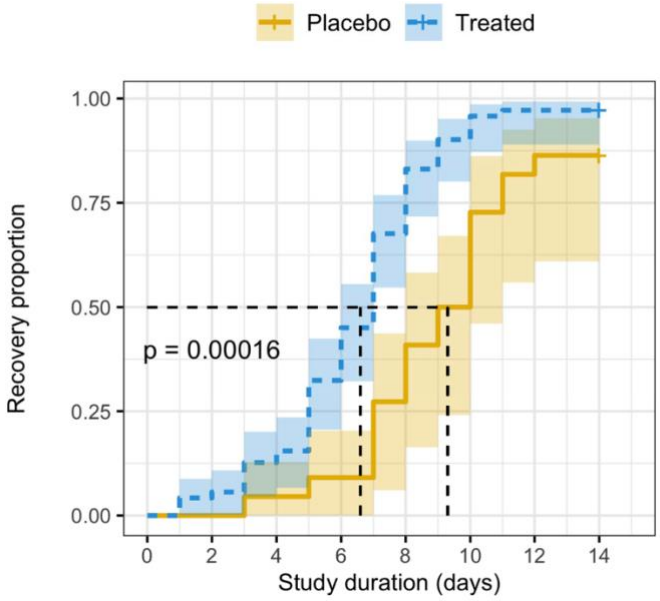


Figure 2. Kaplan-Meier curve for all 93 participants enrolled in the clinical trial that tested a combined metabolic cofactor supplementation in individuals infected with COVID-19.

2.1.4 Pharmacokinetics of Nicotinamide Riboside in Humans

An open-label, non-randomized study of the pharmacokinetics of NR carried out at the University of Washington demonstrated both the impact of NR on NAD^+ levels in humans and its tolerability[16]. Eight subjects underwent dose escalation from 250 mg of NR twice daily to 1000 mg of NR twice daily over the course of nine days, with NR and NAD^+ levels determined at baseline Day 1 and over the course of a 24-hour pharmacokinetic study on Day 9. No adverse events were reported in clinical laboratory values, including potassium, which had been noted to be elevated in previous NR studies. Slight, but significant decreases in hematocrit, hemoglobin, and platelet count were reported. All but one subject showed an increase in blood NR levels from baseline to Day 9, ranging from 10% to 127% increases. Blood NR showed exponential decline from 3 to 12 hours with a half-life of 2.7 hours. Despite relatively fast clearance of NR, blood NAD^+ levels were significantly increased to steady state (see Figure 3) by 35 to 168% from baseline. NR treatment did not produce side effects commonly seen in niacin treatment, such as flushing, pruritus, hyperglycemia, hyperuricemia, or elevation of liver or muscle enzymes. However, a variability in NR bioavailability was observed, suggesting that NR relies on active transport across intestinal mucosa, which can vary highly from person to person.

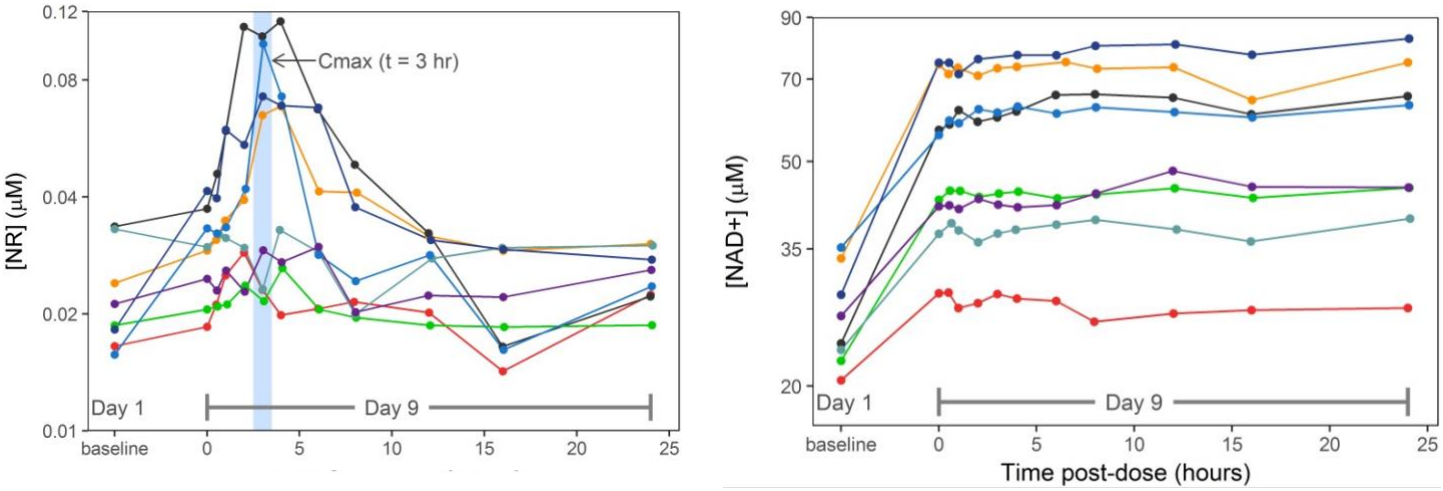


Figure 3. Blood NR and NAD^+ levels in $n = 8$ subjects following NR dose escalation from 250 to 1000 mg twice daily.[6]

2.1.5 Prior Clinical Use of Nicotinamide Riboside

NR has been characterized to be both tolerable and effective in healthy middle-aged and older adults in improving markers of cardiovascular health in healthy middle-aged and older adults. A 12-week randomized, double-blind, single-crossover trial provided 30 healthy subjects with twice daily 600 mg doses of NR for 6 weeks and 6 weeks of placebo (PBO)[12]. Over the course of the study, 14 treatment-emergent adverse events (AEs) were reported in 7 subjects, all of mild severity. During the NR treatment period, AEs included nausea, flushing, leg cramps, and increased bruising. During the PBO period, AEs included headache, skin rash, flushing, fainting, and drowsiness. Only 2 subjects (less than 10%) dropped out of the study because of these AEs, both of whom were in the PBO period. Two out of the three instances of flushing reported also occurred during the PBO period, suggesting the AE is likely unrelated to treatment. No significant changes in clinical labs were observed, including renal function markers and blood lipid profiles. Blood NAD^+ levels increased by 60% from baseline following NR treatment. A five-fold change in nicotinic acid adenine dinucleotide (NAAD) and a 1.5-fold change in a nicotinamide mononucleotide (NMN) were also detected. Adenosine and adenosine triphosphate (ATP) levels also increased following NR treatment, demonstrating its robust impact on metabolism. When all subjects were grouped, mean systolic and diastolic blood pressure was significantly lowered following NR treatment, and carotid-femoral pulse wave velocity, a gold-standard marker of aortic stiffness, was also reduced, although not significantly. NR treatment did not correspond to any significant change in total energy expenditure, oxidative fuel source, physical activity patterns, body mass index, glucose and insulin regulation, and exercise capacity in any subject.

3. SPECIFIC AIMS

3.1 Overall Study Design and Plan

The study uses a double-blinded, placebo lead-in, randomized, parallel group, PBO-controlled design. For this clinical trial, one-hundred individuals over 17 years of age meeting operationalized criteria for Long-COVID will be recruited. These 100 participants will undergo a placebo lead-in period of two weeks. Sixty participants will then be randomized to NR (TruNiagen®, Chromadex) 1000 mg twice daily for 22 weeks, and 40 will receive placebo for the next 10 weeks followed by NR for 10 weeks. Outcomes will consist of standardized cognitive, neuropsychiatric, physical, functional and biomarker assessments as well as daily sleep and activity monitoring with wearable actigraphy.

The study will also help better understand the nature of long-COVID by comparing this group with a healthy control group of individuals who were infected with COVID-19 but fully recovered. To better address this aim, we will also increase our sample size of individuals with long-COVID by testing individuals with long-COVID ages 18 and older who will only complete 1 visit. Comparing these groups will help establish differences in NAD+ levels at baseline associated with long-COVID, as well as help elucidate the cognitive profile, neuropsychiatric and physical functioning, and markers of glia and neural functioning in this population. Participants in these additional cohorts will only complete one visit and will not be administered NR or PBO.

3.2 Study Objectives

The primary objective of the study will be:

1. To measure the effect of NR vs PBO on standard assessments of cognition and subjective cognitive concerns.

The secondary clinical objectives of the study will be:

2. To measure the effect of NR vs PBO on neuropsychiatric symptoms;
3. To measure the effect of NR vs PBO on physical functioning.

The tertiary clinical objective of the study will be:

1. To elucidate how NAD+ levels, cognitive functioning, neuropsychiatric and physical symptoms, and markers of neural injury and inflammation differ between individuals with long-COVID and healthy controls.

The exploratory biomarker objectives of the study will be:

1. To measure the effect of NR on plasma biomarkers of metabolic target engagement and central nervous system (CNS) neural and glial integrity measurable in blood.
2. To measure the effects of NR on cerebral neurophysiology (in an optional sub-study) using resting state functional magnetic resonance imaging (rs-fMRI). Connectivity will be measured with rsBOLD.

3.2.1 Standardized Cognitive, Functional, and Neuropsychiatric Outcome Measures

The primary outcome of this study will be objective measures of cognitive performance and subjective cognitive concerns measured with the following tests:

- The Mini-Mental State Examination (MMSE)[22], Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV) Digit Span backwards[23], Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)[24], the Trail Making Test (TMT)[25], the Delis-Kaplan Executive Function System (DKEFS) Verbal Fluency[26], and the Continuous Performance Test (CPT). [27] Subjective ratings of everyday cognitive functioning will be measured using items from the Behavior Rating Inventory of Executive Function (BRIEF-A)[28] at week 2, week 12 and week 22, and the Everyday Cognition scale (ECog)[29] will be measured at each visit.

The secondary and tertiary clinical and exploratory biomarker outcomes of this study will be:

- Assessment of neuropsychiatric symptoms as measured by:

    - Ratings of mood and anxiety will be measured using the Post Traumatic Stress Disorder Checklist-Civilian (PCL-C)[30], Beck Anxiety Inventory (BAI)[31] and Beck Depression Inventory (BDI)[32]. These standard measures will be administered at each visit.
- Assessment of physical functioning as measured by:
    - The Fatigue Severity Scale[33].
    - Objective daily physical activity levels and sleep data collected using wrist actigraphy (Fitbit Charge 4).
    - Subjective sleep ratings (Pittsburgh Sleep Quality Index)[34] and physical activity (International Physical Activity Questionnaire [IPAQ])[35] measures will be collected using questionnaires at each visit.
- Safety and tolerability will be assessed with adverse event (AE) reporting conducted at every visit.
- Plasma for biomarkers will be collected at Week 2, and at every 5 weeks until the end-of-study, and at the one-time visit for HC and long-covid participants. Plasma biomarkers assays will consist of ultra-sensitive immunochemical measures of proteins reflecting neurodegeneration/neural injury (e.g. NfL, IL-6 and GFAP), as well as NAD+/NADH).
- Cerebral neurophysiology will be measured with functional MRI (optional sub-study).
    - Optional MRI will be conducted at two time points: Week 2 and End of Study (Week 22). Healthy controls will complete the MRI at their one-time visit. Each MRI scan session will include functional MRI with resting state BOLD (rs-fMRI) and pseudo-continuous arterial spin labeling (pCASL) sequences. rs-fMRI measures spontaneous yet synchronized fluctuations in the BOLD signal to determine the strength of functional connectivity within various intrinsic brain networks (i.e., default mode network). pCASL quantifies regional cerebral blood flow using magnetically labeled blood water content and will serve as a marker of resting state cerebral perfusion that has also been shown to correlate with regional brain metabolism and levels of neuronal activity.

3.3 Protocol Adherence

Each investigator must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by Internal Review Board (IRB). Each investigator will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

4. SUBJECT SELECTION

4.1 Inclusion and Exclusion Criteria

4.1.1 Inclusion Criteria

A subject will be eligible for participation in the **clinical trial** if they meet the following:

1. Ages 18 to 65 years;
2. History of SARS-CoV-2 PCR+ or COVID-19 Antigen Rapid Self-Test at least 2 months prior to study entry;
3. SARS-CoV-2 negative (PCR) at study entry;
4. Persistent cognitive difficulties (esp. "brain fog") that began around the time of the acute COVID-19;
5. At least two neurological and/or physical symptoms that started at COVID-19 infection and are ongoing at study entry, including fatigue, weakness, headache, loss of smell, tingling/numbness, shortness of breath, loss of appetite, palpitations/tachycardia, hair loss, musculoskeletal and/or chest pain;
6. Minimum score of 18 on the MMSE;
7. Education level, language skills, and literacy indicates participant is able to complete all assessments;

8. Willing and able to consent, complete all assessment and study procedures.

A subject will be eligible to participate as a **HC Participant** for a one time visit if they meet the following:

1. Ages 18 or older;
2. History of SARS-CoV-2 PCR+ or COVID-19 Antigen Rapid Self-Test+ at least 2 months prior to study entry;
3. SARS-CoV-2 negative (PCR) or COVID-19 Antigen Rapid Self-Test at study entry;
4. Experienced full recovery and does not have persistent cognitive difficulties, neurological and/or physical symptoms associated with COVID-19 infection;
5. Minimum score of 18 on the MMSE;
6. Education level, language skills, and literacy indicates participant is able to complete all assessments;
7. Willing and able to consent, complete all assessment and study procedures.

A subject will be eligible to participate as a **long-COVID Participant** for a one time visit if they meet the following:

1. Ages 18 or older;
2. History of positive SARS-CoV-2 PCR or COVID-19 Antigen Rapid Self-Test at least 2 months prior to study entry;
3. Negative COVID-19 Antigen Rapid Self-Test at study entry;
4. A minimum score of 18 on the Mini-Mental state examination;
5. Report persistent cognitive difficulties ("brain fog") that began around the time of the acute COVID19 illness;
6. At least two neurological and/or physical symptoms that started at COVID-19 infection and are ongoing at study entry, including fatigue, weakness, headache, loss of smell, tingling/numbness, shortness of breath, loss of appetite, palpitations/tachycardia, hair loss, musculoskeletal and/or chest pain;
7. Education level, language skills, and literacy indicates participant is able to complete all assessments;
8. Willing and able to consent, complete all assessment and study procedures.

4.1.2 Exclusion Criteria

A subject will not be eligible for participation in the **clinical trial** if they meet any of the following criteria:

1. Any specific CNS disease history, such as major clinical stroke, brain tumor, normal pressure hydrocephalus, multiple sclerosis, delirium, significant head trauma with persistent neurological of cognitive deficits or complaints;
2. Clinically significant unstable medical condition that could affect safety or compliance with the study and would, in the opinion of the investigator, pose a risk to the participant if they were to participate in the study;
3. Was intubated due to COVID-19;
4. History of neuroimaging with evidence of major infarction, injury, infection, or other focal lesions that may be related to cognitive dysfunction;
5. Major active or chronic unstable psychiatric illness (e.g. depression, bipolar disorder, obsessive compulsive disorder, schizophrenia) within the previous year;
6. Current suicidal ideation;
7. History of alcohol or other substance abuse or dependence within the past two years;
8. Any significant systemic illness or medical condition that could affect safety or compliance with study;
9. Laboratory abnormalities in Vitamin B12, Thyroid Stimulating Hormone (TSH), or other common laboratory parameters that might contribute to cognitive dysfunction or other abnormalities in hematological, hepatic or renal function tests;

10. Current use of medications with psychoactive properties that in the opinion of the principal investigator, may be deleteriously affecting cognition (e.g., anticholinergics, antihistamines, antipsychotics, sedative hypnotics, anxiolytics);
11. Any known hypersensitivity to nicotinamide riboside, or its principal metabolite, nicotinamide mononucleotide;
12. No consumption of dietary supplements containing more than 100mg of niacin, nicotinamide riboside (NR), or nicotinamide mononucleotide (NMN) as the primary agents 30 days prior to baseline and for the duration of the trial.
13. Use of other investigational agents or interventions one month prior to entry and for the duration of the trial;
14. If participating in the optional MRI sub-study: Any contraindication to undergo MRI studies, such as history of cardiac pacemaker or pacemaker wires, metallic particles in the body, vascular clips in the head, prosthetic heart valves, and/or severe claustrophobia impeding ability to participate in an imaging study;
15. Pregnant people or those who are planning to become pregnant within 7 months from study entry.

A subject will not be eligible to participate as a **HC participant** if they meet any of the following criteria:

1. Any specific CNS disease history, such as major clinical stroke, brain tumor, normal pressure hydrocephalus, multiple sclerosis, delirium, significant head trauma with persistent neurological of cognitive deficits or complaints;
2. Clinically significant unstable medical condition that could affect safety or compliance with the study and would, in the opinion of the investigator, pose a risk to the participant if they were to participate in the study;
3. Was intubated due to COVID-19;
4. History of neuroimaging with evidence of major infarction, injury, infection, or other focal lesions that may be related to cognitive dysfunction;
5. Major active or chronic unstable psychiatric illness (e.g. depression, bipolar disorder, obsessive compulsive disorder, schizophrenia) within the previous year;
6. Current suicidal ideation;
7. History of alcohol or other substance abuse or dependence within the past two years;
8. Any significant systemic illness or medical condition that could affect safety or compliance with study;
9. Current use of medications with psychoactive or other properties that in the opinion of the principal investigator, may be deleteriously affecting cognition (e.g., anticholinergics, antihistamines, antipsychotics, sedative hypnotics, anxiolytics);
10. If 65 years of age or younger, have any contraindication to undergo MRI studies, such as history of cardiac pacemaker or pacemaker wires, metallic particles in the body, vascular clips in the head, prosthetic heart valves, and/or severe claustrophobia impeding ability to participate in an imaging study;
11. Pregnant individuals.

A subject will not be eligible to participate as an **long-COVID Participant** if they meet any of the following criteria:

1. Any specific CNS disease history, such as major clinical stroke, brain tumor, normal pressure hydrocephalus, multiple sclerosis, delirium, significant head trauma with persistent neurological of cognitive deficits or complaints;
2. Clinically significant unstable medical condition that could affect safety or compliance with the study and would, in the opinion of the investigator, pose a risk to the participant if they were to participate in the study;
3. Was intubated due to COVID-19;

4. History of neuroimaging with evidence of major infarction, injury, infection, or other focal lesions that may be related to cognitive dysfunction;
5. Major active or chronic unstable psychiatric illness (e.g. depression, bipolar disorder, obsessive compulsive disorder, schizophrenia) within the previous year;
6. Current suicidal ideation;
7. History of alcohol or other substance abuse or dependence within the past two years;
8. Any significant systemic illness or medical condition that could affect safety or compliance with study;
9. Current use of medications with psychoactive or other properties that in the opinion of the principal investigator, may be deleteriously affecting cognition (e.g., anticholinergics, antihistamines, antipsychotics, sedative hypnotics, anxiolytics);
10. If 65 years of age or younger, have any contraindication to undergo MRI studies, such as history of cardiac pacemaker or pacemaker wires, metallic particles in the body, vascular clips in the head, prosthetic heart valves, and/or severe claustrophobia impeding ability to participate in an imaging study;
11. Pregnant individuals.

4.1.2.1 Individuals of Childbearing Age

Individuals of childbearing age will be eligible for the study. However, pregnant individuals will be ineligible for the clinical trial and sub-studies studies, and those who are planning to become pregnant within 7 months from study entry will be ineligible for the clinical trial.

4.2 Recruitment

Approximately 120 subjects with long-COVID will be screened, and after screening, approximately 100 subjects are expected to complete the clinical trial. Approximately 75 healthy controls (50 participants ≤65 years of age, and 25 participants >65 years of age) and 75 long covid participants (50 participants <65 years of age, and 25 participants ≥65 years of age) are expected to complete the one-time visit. All subjects in the long-COVID group participating in the clinical trial will be given the option to participate in the optional repeat MRI sub-study until 24 subjects have completed the sub-study, and 50 more can complete the baseline MRI. All 50 healthy controls and 50 long-COVID participants ≤65 years of age are required to complete the MRI at their one-time visit. Individuals older than 65 years will not complete an MRI.

4.2.1 Recruitment of Subjects through Advertising

Advertisement flyers will be posted on bulletin boards around MGH (both the main campus and Charlestown campus) to advertise for the study as well as ad advertisement on Partners Rally for Research. Advertisement flyers will also be provided at community events (e.g., health fairs) and other institutions (e.g., churches, community centers). An email address and phone number will be provided that will ring directly to the research coordinator, and voice messages can be left for the coordinator on a password-protected voice mailbox. Additionally, for participants collected through Rally, we will email them a survey to complete via REDCap using send secure. This digital survey will allow us to assess their eligibility. Once participants submit the survey and are deemed eligible, we will contact them by phone to ask any follow-up questions, collect more information as needed, and schedule them for an in-person visit. We may still pre-screen people by phone if the person communicates that this is their preferred method.

4.2.2 Recruitment of Subjects Identified through Private Medical Information

The Neuro-Infectious Diseases Unit, Neurology Clinic, Psychological Assessment Center, Multicultural Assessment and Research Center, MGH Primary Care clinics, the NeuroScience dashboard, and Neuropsychiatric Clinic physicians will be made aware of the study, including eligibility criteria. If they feel

that a patient of theirs may be a candidate for the study, they will obtain the patient's permission to be contacted by the study staff or provide a flyer with the study's information. Those who agree to be contacted will be contacted by the study coordinator who will explain the study in further detail and if the subject is interested, potentially complete a telephone or online prescreening survey.

4.2.3 Recruitment of Subjects from the MGH McCance Center for Brain Health

Some subjects will be recruited from the study titled "Brain Health in COVID-19 survivors and their caregivers" at the MGH McCance Center for Brain Health (IRB protocol 2020P001455).

4.2.4 Recruitment of Subjects from Among the Investigators' Own Patients

Dr. Steven Arnold (co-investigator) is a neurologist in the MGH Memory Disorders Unit and Dr. Guzmán-Vélez a neuropsychologist at the Multicultural Assessment and Research Center. Subjects may also be recruited from among their patients. Special care will be taken to ensure patients are aware that their participation is completely voluntary and that their decision to participate will not affect their care. Dr. Arnold and Dr. Guzmán-Vélez will obtain the patient's permission to be contacted by the study staff or provide a flyer with the study's information. If interested, the patient will be contacted by the study coordinator who will explain the study in further detail and if the subject is interested, potentially complete a telephone or online prescreening survey.

5. SUBJECT ENROLLMENT

5.1 Informed Consent Process

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations, and ICH Guidance Documents pertaining to informed consent.

Potential subjects will be given general information about the research (e.g., through informational sheets, letters, or discussion with their treating physicians). If they are interested in learning more about the study, they will then contact the research coordinator. The research coordinator will obtain verbal consent in accordance with Partner's Prescreening Guidelines prior to performing a telephone prescreening interview. Subjects who meet pre-screening criteria for the clinical trial and wish to continue the screening process will be scheduled for a screening visit that will take place in person at MGH CNY campus. Subjects who are eligible as HC or long-COVID participants based on the phone screening will be scheduled for their one-time visit that will take place in person at MGH CNY campus.

At the clinical trial's screening visit and the one time visit for the HC and long-COVID participants, the investigator will meet with the potential subject to review and discuss the details of the study using the informed consent document as a guide. A copy of the informed consent document will be sent to the subject before the first visit takes place, and they will have at least 24 hours between when the document is received and when the first visit is scheduled in order for the subject to have adequate time to fully review the Informed Consent Document.

The informed consent process will be conducted using a REDCap-based electronic consent form. The consent form has been developed in REDCap, a secure, web-based, HIPAA-compliant, data collection platform with a user management system allowing project owners to grant and control varying levels of access to data collection instruments and data for other users. If requested by the subject, a traditional hard copy consent form will be provided to the subject along with any materials needed for return shipping.

During the first visit, the informed consent document will be reviewed with the participant by a licensed clinician investigator. The potential subject may ask to speak with the physician investigator should a non-physician investigator be involved in obtaining informed consent. The informed consent discussion will include all of the required elements of informed consent, including the purpose of the research, the procedures to be followed, the risks and discomforts, as well as potential benefits associated with participation, and alternative procedures to study participation. Their questions will be answered to their satisfaction. The subject will be provided with adequate time to reflect on the potential benefits and risks and possible discomforts of participation, and to make an informed decision.

If the subject agrees to participate in the study, a link will be sent to the REDCap e-consent form or the subject will sign the provided hard-copy informed consent form. For REDCap e-consent, subject signatures will be obtained using electronic signature via mouse followed by electronic signature by the doctorate-level clinician investigator. The signed hard-copy consent forms will then be signed by the consenting investigator. Upon completion of the informed consent process, subjects will be provided with a hard copy of the signed consent from and hard copies will be stored in each subject's binder.

All subjects must be deemed capable of providing informed consent by the Investigators. Subjects who are not deemed capable of providing informed consent will be excluded from this study.

5.2 Remuneration

Subjects for the clinical trial will receive $20 for their participation in each visit. If subjects elect to participate in MRIs, they will receive $100 for each MRI. Subjects who are part of the clinical trial will also receive $20 upon completion of the study as long as they were at least 80% compliant with all study activities, including taking their medication, completing cognitive tests, and completing the Fitbit activity tracking. Therefore, if a subject elects to undergo all additional procedures, the maximum amount a subject would be eligible to receive is $320 at study completion. If a subject does not elect to participate in any optional procedures but is compliant with study tasks, they would be eligible to receive $120. We anticipate some subjects may come from outside the region and if so, appropriate travel expenses may be reimbursed including transportation up to $15 per visit. To minimize the inconvenience of frequent visits, meal and parking vouchers for use at the MGH Charlestown Navy Yard (CNY) facilities will also be offered at each visit.

Long-COVID and HC participants who are 65 years of age or younger will receive $120 for their participation in the one-time visit. If they live outside the region, appropriate travel expenses may be reimbursed including transportation up to $15 for the visit. Meal and parking vouchers for use at the MGH CNY cafeteria and parking garage will also be offered.

Long-COVID and HC participants who are older than 65 years of age, we will pay $80 for their participation in the one-time visit. If they live outside the region, appropriate travel expenses may be reimbursed including transportation up to $60 for the visit. Meal and parking vouchers for use at the MGH CNY cafeteria and parking garage will also be offered.

5.3 Randomization

Subjects in the clinical trial will be randomized into one of two groups (see Figure 4 below). Randomization will be done by MGH Clinical Trials Pharmacy.

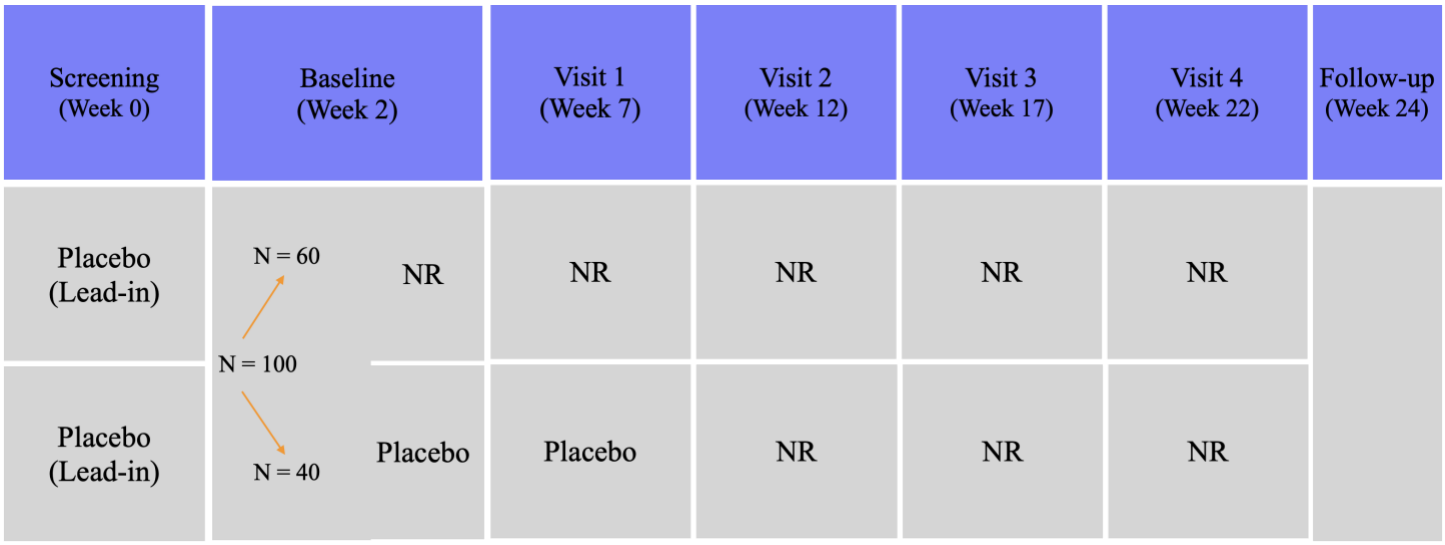


Figure 4. Study Schema

5.4 Reasons for Withdrawal

A study subject will be discontinued from participation in the study if:

- Any clinical adverse event (AE), concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets any exclusion criteria (either newly developed or not previously recognized).
- Tests positive for SARS-CoV-2 PCR or COVID-19 Antigen Rapid Self-Test
- The subject is less than 80% or greater than 120% compliant with NR dosing.

Subjects are free to withdraw from participation in the study at any time upon request.

5.4.1 Handling of Withdrawals

A subject may choose to discontinue participation in the study at any time. An Early Termination visit will occur when a subject withdraws consent, i.e. withdrawing his or her participation in future study procedures.

5.5 Termination of Study

This study may be prematurely terminated if, in the opinion of the investigator, there is sufficient reasonable cause.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Unsatisfactory enrollment.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.

If the study is prematurely terminated or suspended, the investigators will promptly inform the institution and regulatory authorities of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC will also be informed promptly and provided the reason(s) for the termination or suspension by the investigator, as specified by applicable regulatory requirement(s).

6. STUDY PROCEURES

6.1 Schedule of Assessments for the clinical trial

Activity (n=100)	Week 0	Week 2	Week 7	Week 12	Week 17	Week 22	Early DC	Week 24
	Screening/Lead-In	Baseline	Visit 1	Visit 2	Visit 3	Visit 4		Phone Follow Up
Informed Consent[1]	X							
Demographics	X							
Medical & COVID-19 History/ Safety Labs[2,3]	X							
Long COVID-19 symptoms assessment	X	X	X	X	X	X	X	X
SARS-CoV-2 PCR test	X							
Height and Weight	X			X		X	X	
Vital Signs	X	X	X	X	X	X	X	
Inclusion/ Exclusion Review[4]	X	X	X	X	X	X		
Physical and Neurological Examination[5]	X			X		X	X	
MMSE	X			X		X	X	
Concomitant Medications and Supplements[4]	X	X	X	X	X	X	X	X
Randomization	X							

Dispense NR/PBO[4]	X	X	X	X	X			
Accountability and Compliance		X	X	X	X	X	X	
AE Reporting[4,6]		X	X	X	X	X	X	X
Office Cognitive Assessment[7]		X		X		X	X	
Neuropsychiatric Assessment[8]	X	X	X	X	X	X	X	
Office Functional Assessment		X		X		X	X	
Blood Collection for PK and Biomarkers[9]		X	X	X	X	X	X	
Subjective cognitive concerns assessment[10]	X	X	X	X	X	X	X	
Physical activity Assessment[11]	X	X	X	X	X	X	X	
Sleep Assessment[12]	X	X	X	X	X	X	X	
Daily Activity Assessment[12]	_____ongoing_____					ongoing___X		

6.2 Schedule of Assessments for the one-time visits

Activity	Healthy Control (n=75)	Long-COVID Participant (n=75)
	One Time Visit	One Time Visit
Informed Consent[1]	X	X
Demographics	X	X

Medical & COVID-19 History	X	X
Long COVID-19 symptoms assessment	-	X
SARS-CoV-2 PCR test	X	X
Height and Weight	X	X
Vital Signs	X	X
Inclusion/ Exclusion Review[4]	X	X
SMMSE	X	X
Concomitant Medications and Supplements[4]	X	X
AE Reporting[4,5]	X	X
Office Cognitive Assessment[6]	X	X
Neuropsychiatric Assessment[7]	X	X
Office Functional Assessment	X	X
Blood Collection for PK and Biomarkers[8]	X	X
Subjective cognitive concerns assessment[9]	X	X
Sleep Assessment[11]	X	X

[1]No study procedures apart from the telephone pre-screening visit will be performed prior to the signing of the informed consent form (ICF). All subjects will sign an ICF prior to undergoing any study tests or procedures.
[2]After initial assessment of medical history during the screening period, updated medical history will be collected in subsequent visits.
[3]Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, B12 and TSH, as well as a pregnancy test to women of childbearing potential.
[4]These procedures can take place virtually via Zoom videoconference if necessary.

[5]All adverse events that occur AFTER signing consent form until the end of the follow up visit will be included.
[6]Including D-KEFS Verbal Fluency, Trail Making Test, RBANS, WAIS-IV Digit Span Backwards, BRIEF-A, CPT.
[7]Including BDI, BAI, PCL-C
[8]Including collection of dried blood spot samples
[9]ECog
[10]IPAQ
[11]Pittsburgh Sleep Quality Index
[12]Fitbit Charge 4 Wearable Device

6.2.1 Screening/Lead-In Visit (Week 0)

The screening procedures will determine the subject's eligibility for the study and will take approximately 3 hours.

The following procedures will take place at the Screening/Lead-In visit:

- Obtain written informed consent from the subject.
- Obtain demographics and medical history, including assessment of COVID-19 symptoms
- Complete SARS-CoV-2 PCR test
- Safety Labs
- Perform physical and neurological examination
- Measure Vital Signs
- Measure height and weight
- Review and document concomitant medications, supplements, and therapies
- Administer the MMSE
- Provide Fitbit
- Administer questionnaires about neuropsychiatric symptoms, sleep, physical activity, and others.
- Dispense PBO

6.2.1.1 Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening, *at a minimum,* the following information should be captured and entered into the Electronic Data System (EDC).

- Inclusion/Exclusion Criteria
- Demographics
- Reason for screen failures

6.2.2 Baseline Visit (Week 2)

The following procedures will be performed and will take approximately 4-5 hours depending on participation in the MRI sub-study. If the subject has elected to participate in the MRI sub-study, the visit may occur over multiple days as long as all tasks are completed during the study visit window.

- Measure Vital Signs
- Blood draw and a collect dried blood spot sample for biomarker analysis
- Assess study supplement compliance
- Assess COVID-19 related symptoms
- Administer cognitive assessments
- Administer questionnaires about neuropsychiatric symptoms, sleep, physical activity and others
- Assess and document AEs and changes to medical history[1]
- Review and document concomitant medications, supplements, and therapies[1]
- Dispense study supplement or PBO[1]
- Perform optional MRI scan
- Reassess inclusion and exclusion criteria[1]

[1]These procedures can take place virtually via Zoom videoconference if necessary.

6.2.3 Follow Up Visits (Weeks 7, 12 and 17)

These visits will take place every 5 weeks (± 2 days) following Baseline.

- Review and document concomitant medications, supplements, and therapies[1]
- Assess COVID-19 related symptoms
- Perform physical assessments
- Perform physical and neurological examination
- Measure vital signs
- Dispense study supplement or PBO[1]
- Assess study supplement compliance
- Administer battery of cognitive assessments (only week 12)
- Administer questionnaires about neuropsychiatric symptoms, sleep, physical activity and others
- Blood draw and a collect dried blood spot sample for biomarker analysis
- Assess and document adverse events (AEs) and changes to medical history[1]

[1]These procedures can take place virtually via Zoom videoconference if necessary.

6.2.4 Final study visit

This visit will take place at Week 22.

- Measure Vital Signs
- Perform physical and neurological examination
- Blood draw and a collect dried blood spot sample for biomarker analysis
- Assess study supplement compliance
- Assess COVID-19 related symptoms
- Administer cognitive assessments
- Administer questionnaires about neuropsychiatric symptoms, sleep, physical activity and others
- Assess and document AEs and changes to medical history[1]
- Review and document concomitant medications, supplements, and therapies[1]
- Perform physical assessments
- Perform optional MRI scan

[1]These procedures can take place virtually via Zoom videoconference if necessary.

6.2.5 Early Termination Visit

If the subject withdraws from the study before completion of all study visits, they will be invited to participate in an Early Termination Visit within 7 days of stopping treatment.

- Measure Vital Signs
- Perform physical and neurological examination
- Blood draw and a collect dried blood spot sample for biomarker analysis
- Assess study supplement compliance
- Assess COVID-19 related symptoms
- Administer cognitive assessments
- Administer questionnaires about neuropsychiatric symptoms, sleep, physical activity, and others
- Assess and document AEs and changes to medical history[1]
- Review and document concomitant medications, supplements, and therapies[1]
- Perform optional MRI scan

[1]These procedures can take place virtually via Zoom videoconference if necessary.

6.2.6 HC One Time Visit

Healthy control subjects will only complete one visit.

- Obtain written informed consent from the subject.
- Obtain demographics and medical history, including assessment of COVID-19 symptoms
- Complete SARS-CoV-2 PCR test or a rapid antigen test
- Measure Vital Signs
- Measure height and weight
- Administer the MMSE
- Administer questionnaires about neuropsychiatric symptoms, sleep, physical activity and others.
- Blood draw and a collect dried blood spot sample for biomarker analysis
- Administer cognitive assessments
- Perform MRI scan (if participant is ≤65 years of age)

6.2.7 Long-COVID One Time Visit

Long-COVID participants will only complete one visit.

- Obtain written informed consent from the subject.
- Obtain demographics and medical history, including assessment of COVID-19 symptoms
- Complete SARS-CoV-2 PCR test or a rapid antigen test
- Measure Vital Signs
- Measure height and weight
- Administer the MMSE
- Administer questionnaires about neuropsychiatric symptoms, sleep, physical activity and others.
- Blood draw and a collect dried blood spot sample for biomarker analysis
- Administer cognitive assessments
- Perform MRI scan (if participant is 65 years of age or younger)

6.2.8 Protocol Deviations

A protocol deviation is any noncompliance with the current clinical trial protocol. The noncompliance may be on the part of the subject, the PI, or the study staff. As a result of deviations, corrective actions will be developed by the PI and implemented promptly. All deviations from the protocol must be addressed in the subject's documents. Protocol deviations will be sent to the IRB per their guidelines and entered in the Protocol Deviations Log in the EDC System.

6.2.9 Missed Visits and Procedures

Missed visits and any procedures not performed (not attempted) for reasons other than illness or injury will be reported as a protocol deviation. Procedures or visits not performed due to illness, injury, or disability, including procedures that were attempted but failed (i.e., weight unable to be obtained due to subject immobility) will not be reported as protocol deviations. Participants who are unable to attend their visit within the approved 3-day window will receive overnight-shipped extension supplements before their original supply runs out. This allows them to maintain their supplement regimen until they can come in for their visit. We will confirm their mailing address before we send the supplement and provide the tracking number. Signature will be required upon delivery. We will also contact them on the date that they receive the supplement to confirm that it arrived in perfect condition.

7 CLINICAL ASSESSMENTS AND OUTCOME MEASURES

7.1 Clinical Variables

Assessments will be performed at designated time-points throughout the study for clinical evaluation. In addition to the assessments evaluated below, subjects will provide information on their demographics, past medical and COVID-19 history, family history, and concomitant medication usage.

7.1.1 Safety Measures

7.1.1.1 Vital Signs, Height, Weight

Vital signs, including systolic and diastolic blood pressure, pulse rate (radial artery)/minute, respiratory rate/minute, and temperature will be assessed at specified visits. Verbal weight may be documented for those subjects utilizing a wheelchair.

7.1.1.2 Clinical Laboratory Assessments

Study participants in the clinical trial will be asked to provide approximately 20 mL of blood for safety lab analysis at screening. The participant will have his or her whole blood collected by either a nurse or phlebotomist from a peripheral vein. Blood will be handled, processed, and analyzed in accordance with regulations set forth by the American Society for Clinical Pathology and the College of American Pathologists.

The investigator may order additional testing, if thought to be necessary, to further assess an adverse event (AE).

7.1.1.3 Adverse Events

Once the informed consent form has been signed by the subject, inquiry about all adverse events (AEs) will be performed at each study visit. The AE grading system being used will be the CTCAE protocol.

7.1.2 Clinical Assessments

7.1.2.1 Neuropsychiatric and Functional Assessments

7.1.2.1.1 Beck Anxiety Inventory (BAI)

The Beck Anxiety Inventory[31] (BAI) is a 21-item, self-administered questionnaire that measures the presence and severity of anxiety in psychiatric populations. It was constructed to avoid confounds due to comorbidity of depression, and it has been found to be highly valid, reliable, and consistent[23]. The BAI lists 21 common symptoms of anxiety and asks the subject to rate the severity with which they experience each symptom (not at all, mildly, moderately, or severely). Scores of 0-21, 21-35, or 36-63 indicate low, moderate, or severe anxiety, respectively.

7.1.2.1.2 Beck Depression Inventory (BDI)

The Beck Depression Inventory[32] (BDI) is a widely used assessment of depression severity used in clinical and research settings. It is a 21-item, self-administered rating inventory with a mean internal consistency of 0.86. The BDI ranks responses to several domains of depression, including mood changes, guilt, self-image, suicidality, affect, and apathy on a scale from 0 to 3 and provides an assessment of the level of depression, spanning minimal to mild to moderate to severe depression.

7.1.2.1.3 Post Traumatic Stress Disorder Checklist – Civilian (PCL-C)

The Post Traumatic Stress Disorder Checklist – Civilian (PCL-C)[30] is a standardized self-report measure of post-traumatic stress disorder (PTSD) that was designed to be applied to any traumatic event. The PCL-C is comprised of 17 items that correspond to key symptoms of PTSD as established by the Diagnostic and Statistical Manual of Mental Disorders (DSM).

7.1.2.1.4 Behavior Rating Inventory of Executive Function (BRIEF-A)

The Behavior Rating Inventory of Executive Functioning (BRIEF-A)[28] is a standardized rating scale to assess self-regulation and executive function in everyday behaviors. It is a 75-item scale including a Behavioral Regulation Index consisting of inhibition, shifting, emotional control, and self-monitoring scales and a Metacognition Index consisting of initiation, working memory, planning/organization, task monitoring, and organization of material scales. This study will only use the BRIEF-A self and not the informant report.

7.1.2.1.5 Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index[34] is a self-report questionnaire that assesses sleep quality over a 1-month time interval. It consists of 19 individual items that are usually completed between 5 to 10 minutes.

7.1.2.1.6 International Physical Activity Questionnaire (IPAQ)

The International Physical Activity Questionnaire[35] is a self-reported measure of physical activity that can be administered by telephone interview or self-administration. The IPAQ collects information on time spent walking, in vigorous- and moderate-intensity activity and in sedentary activity. The IPAQ has been attributed reasonable measurement properties for monitoring population levels of physical activity among 18- to 65- years old adults in diverse settings.

7.1.2.2 Neurocognitive Assessments

7.1.2.2.1 Mini-Mental State Examination (MMSE)

The Mini-Mental State Examination (MMSE)[22] is a widely used cognitive screening measure that includes items that measure orientation, attention, memory, language, and visuo-spatial abilities. Scores range from 1 to 30, with scores greater than 25 being considered normal.

7.1.2.2.2 Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)[24] is a commonly used 25-minute, standardized neurocognitive battery assessing functioning across multiple cognitive domains. It is designed to be administered repeatably, with several equivalent alternate forms available. The RBANS measures a total of five neurocognitive domains using twelve subtests: immediate memory (List Learning and Story Memory), visuospatial/constructional (Figure Copy and Line Orientation), language (Picture naming and Semantic Fluency), attention (Digit Span and Coding), and delayed memory (List Recall, List Recognition, Story Memory, and Figure Recall). The RBANS will be administered at Baseline, Week 12 , and End of Study (Week 22) using alternate forms.

7.1.2.2.3 Trail Making Test (TMT)

The Trail-Making Test (TMT)[25] is a graphomotor letter and number-letter sequencing task that will be administered to test processing speed, mental flexibility, and set-switching. The test takes approximately 5 minutes to complete.

7.1.2.2.4 Wechsler Adult Intelligence Scale – 4th edition (WAIS-IV) Digit Span

Wechsler Adult Intelligence Scale – 4th edition (WAIS-IV) Digit Span[23] is a verbal measure of attention and working memory that consists of three tests: 1) digits forward, which requires repeating a string of numbers; 2) digits backwards, which requires repeating a string of numbers backwards; and 3) and digit sequencing, which requires organizing a string of numbers from lowest to highest.

7.1.2.2.5 Delis-Kaplan Executive Function System (DKEFS) Verbal Fluency

The Delis-Kaplan Executive Function System (DKEFS) Verbal Fluency[26] is a measure of executive functioning that is composed of three different conditions: 1) phonetic fluency, which requires listing as many words as possible that begin with a specific letter in 60 seconds; 2) semantic fluence, which requires listing as many animals as possible in 60 seconds; and 3) category switching requires switching between two semantic categories in 60 seconds.

7.1.2.2.6 Everyday Cognition Scale (ECog)

The Everyday Cognition Scale (ECog)[29] is a self-report questionnaire that assesses a global factor and six domain-specific factors associated with everyday function and cognition (e.g. memory, planning, organization, language, etc). The ECog has been shown to be correlated with objective neuropsychological tests.

7.1.2.2.7 Continuous Performance Test (CPT)

The Continuous Performance Test is a test of sustained attention and alertness in which subjects are presented with a string of letters[27]. The subject is instructed to press a button every time they see an X and not to press it for any other letter. Letters are presented for five minutes. The subject is then given a brief break before continuing to the next part of the study, the AX task. In this section, the subject is told to press the button when they see an X that follow right after an A but not when there is an X that is not preceded by an A. The subject is then presented with another five-minute block of letters. This section of the task is also a test of inhibition and executive control. Task administration will utilize Millisecond Inquisit software, a computerized stimulus presentation platform optimized for temporally precise stimuli presentation and reaction time recording of psychological assessments. The CPT will be administered at Baseline, Week 12 , and End of Study (Week 22). Inquisit Web requires only a subject code and no personally identifiable information.

7.1.2.3 Physical Functioning Assessment

7.1.1.3.1 Physical and Neurological Examination

A physical and neurological examination will be performed at the Baseline visit, Visit 2, and End of Study (Week 22). The following systems will be examined: general appearance, head, eyes, ears, nose, throat, neck, chest, heart, abdomen, extremities, edema, peripheral vascular, skin and appendages, musculoskeletal, central nervous system, muscles and back. COVID-19 related symptoms will be measured using the COVID-19 checklist and the C19-YRS rehabilitation screening tool. HC and long-COVID participants will not undergo a physical and neurological examination.

7.1.2.4 Phlebotomy and collection of dried blood spot samples for Biomarkers

Subjects will provide additional blood samples for biomarker analysis from Baseline to End of Study visits, and HC and long-COVID participants during their one-time visit. Biomarker analysis will include NAD+/NADH, cytokines (e.g. IL-6), oxidative stress marker 8-OHdG), Irisin; NfL, Aβ42, Aβ40, tau, GFAP, UCH-L1, and other analytes. At each visit (Weeks 0-22), 20 ml (1.3 tablespoons) of blood will be collected for these analyses,

for a total of 120 ml (7.8 tablespoons) over the course of the study. Subjects will also provide a dry blood spot sample with a finger prick to measure the concentration of NAD+ from Baseline to End of Study visits.

7.1.2.5 Magnetic Resonance Imaging (MRI)

Subjects will be given the opportunity to participate in an optional MRI sub-study at Baseline (Week 0) and End of Study (Week 22). Subjects in the healthy control group will complete the MRI at their one-time visit. Subjects electing to participate will be asked to complete both MRIs to be part of the sub-study. Data will be collected on a 3-Tesla Siemens scanner with the following scan sequences: a high resolution T1-weighted scan, T2 FLAIR, resting state BOLD MRI, and pCASL at rest.

Resting state BOLD measures low-frequency spontaneous fluctuations in BOLD signal across time, where correlations of this spontaneous activity between brain regions are thought to reflect functional connectivity. The 10-minute rsfMRI scan will be acquired with whole-brain echo planar imaging (EPI) time series with a voxel size of 2x2x2 mm isotropic, TR/TE = 4000/30 ms, acquisition matrix = 64x64, and 40 axial slices. Subjects will be instructed to keep their eyes open throughout the duration of the scan. rsfMRI data will be processed within the FreeSurfer FS-FAST stream using standard pre-processing steps including motion correction, masking of non-brain tissue, registration to the anatomical high-res image, sampling to the surface, and surface smoothing by 5 mm. Temporal filtering to extract signals in the 0.008-0.08 Hz frequency band will be performed, followed by linear regression to factor out mean global signal, average signal from white matter, and average signal from ventricular cerebrospinal fluid (CSF) regions. To perform functional connectivity analyses, we will use a seed-based approach to examine temporal correlations between the mean time course within anatomically-defined nodes and the rest of the brain.

ASL-MRI is another indicator of brain function, quantifying regional cerebral blood flow, which in turn is linearly coupled to regional brain metabolism and neuronal activity[36]. It is especially well suited to drug trials in which change from baseline is of prime interest. Pseudo-Continuous ASL (pCASL), which provides excellent sensitivity and temporal stability[37], will be used in the protocol. The single shot pCASL scan will be acquired with the following parameters: 24 cm field of view, 2x2x2 mm isotropic, TR/TE = 4300/3.2 ms, acquisition matrix = 64x64, slice thickness = 6mm, temporal bolus = 1800 ms with a 1800 ms post labeling delay. Corresponding field map scans will also be acquired to correct for blurring and signal dropout during image processing. All data processing and analysis will utilize tools available through FSL's Bayesian Inference for Arterial Spin Labeling MRI (BASIL) toolbox.

7.1.2.6 SARS-CoV-2 PCR

The CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay will be used to measure active COVID-19. During the test, the subject is asked to tilt the head back 70 degrees. While gently rotating the swab, a 6-inch swab is inserted less than one inch into nostril (until resistance is met at turbinates).

7.1.2.7 The Fatigue severity scale

The fatigue severity scale[33] is a measure of fatigue that contains 10 items. The subjects will be instructed to respond to each statement using a scale of 1 to 7, with 1 indicating "Strongly Disagree" and 7 indicating "Strongly Agree."

7.1.3 Daily Outcomes

7.1.3.1 Daily activity and sleep tracking

Each subject will be provided with a Fitbit Charge 4 that they will wear throughout the study. The device will track the participant's heart rate, activity, and sleep. The subject will be instructed to wear their device at all times, except for when it is being charged and when they are bathing. HC and long-COVID participants will not be given a FitBit.

7.1.3.1.1 Charging and syncing procedures

Subjects will be instructed to ensure that the device is charged at all times. The subject will be instructed to charge their device when their battery is low (roughly once every 4 days) during a period of inactivity, such as when watching TV. The subject will be explicitly instructed not to charge their device during times of sleep. The subject will be instructed to sync their device at least 3 times per week. Charging and syncing compliance will be routinely monitored by a study coordinator. The coordinator will conduct periodic reviews of the subject's charging and syncing compliance by logging onto the subject's Fitbit dashboard. If the device has not been appropriately charged or synced, the study coordinator will contact the subject to remind them to sync or charge their device as needed. If a subject requires more than three such reminders, they will be considered non-compliant, and the PI will determine if the subject should continue in the study or if their study participation should be discontinued.

7.2 Training and Validation

All evaluators must be certified by the study PI to perform cognitive and psychiatric outcome assessments. It is strongly preferred that a single evaluator performs all measures with a given instrument throughout the study, if possible.

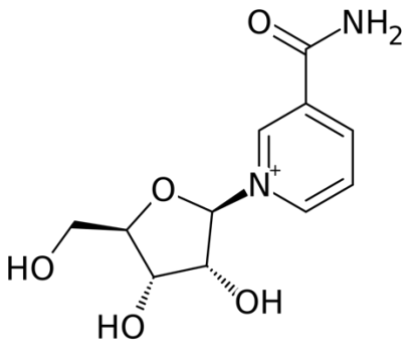
8. INTERVENTIONS ADMINISTERED

8.1 INTERVENTION

8.1.1 Study Product Description

Niagen® is a commercially available form of nicotinamide riboside (NR) manufactured by ChromaDex, Inc. Niagen® is generally recognized as safe (FDA GRAS Notice No. 635) for use in a variety of media, including vitamin waters, protein shakes, and powdered beverages. Niagen® has five issued process and use patents in the United States and several others internationally.

Chemical Structure of nicotinamide riboside:



8.1.2 Drug and Placebo

Chromadex Inc. will provide 250 mg capsules. Identical appearing capsules with only filler will be prepared as placebo.

8.1.3 Acquisition
When the study intervention shipment arrives at the site, the person accepting the shipment must perform an inventory and fill out a supplement receipt log. The contents of the shipment must be counted, verified, and inspected for damage. Any damaged shipments will be documented. The study supplement will be picked up from MGH Clinical Trials Pharmacy on the same day it will be dispensed. Detailed logs will be kept recording quantity of supplement dispensed and study staff in charge of supplement transport and dispensing.

8.2. Product Storage and Stability
Niagen NR is found to be stable under ambient conditions of 25 °C and 60% relative humidity for 11 months in a solid, powdered form.

8.3 Dosage, Preparation and Administration of Study Intervention
Niagen NR 1000 mg (four capsules) or PBO (four capsules) should be taken orally twice daily with food. The capsules should be swallowed whole.

8.4 Modification of Study Intervention for a Subject
Dosing may be suspended at any time by the PI or designated licensed clinician sub-investigator. This will be documented along with the reason(s) and dates of adjustment in the CRF for each subject requiring this manipulation. The PI or designated licensed clinician Sub-Investigator may suspend the dosage of study dietary supplement or discontinue the study supplement for AEs thought to be related to the study supplement or for other reasons during the trial (the reason and dates of suspension or dose suspension must be documented in CRF). If the AE is mild or moderate, the dosage may be re-started after the event improves. If the AE is serious or life threatening and deemed to be definitely related to the dietary supplement, the study dietary supplement will be discontinued immediately. Study subjects must remain off the study dietary supplement permanently. Subjects may not resume study dietary supplement. All AEs will be followed to resolution.

8.4.1 Dosage Discontinuation
Reasons for discontinuation of study dietary supplement may include an AE or PI recommendation, protocol deviation, loss-to-follow-up, patient request, or death. All serious adverse events (SAEs) that occur in a subject who has discontinued early must be recorded and reported within 24-hours of awareness. Study subjects who discontinue study dietary supplement prematurely (early discontinuation from study) and decide not to remain in the study will be encouraged to return for an Early Termination Visit.

8.5 Assessment of Subject Compliance
Subjects will be instructed to return empty and unused study dietary supplement containers at each clinic visit or the Early Discontinuation Visit. Research staff will review returned and unused study dietary supplement and log information into the reconciliation form to determine compliance. Non-compliance will be defined as taking less than 80% or more than 120% of study agent as determined by unused capsule counts. If a study subject is non-compliant with study dietary supplement, research staff should re-educate and train the subject in administration of study agent. If the subject's non-compliance persists, it will be left up to the PI to determine whether the subject should be discontinued from the study.

9. STATISTICAL ANALYSIS

Descriptive statistics will be used to characterize the cohort's demographic and clinical variables. We will conduct independent sample t-tests to examine between-group differences in demographic variables at baseline and analysis of variation (ANOVA) to test for between-group differences in treatment compliance for each study visit.

9.1 Primary analysis

For all outcomes, we will perform intention-to-treat (ITT) analysis and mixed-effects model repeated measures (MMRM). Group (NR-NR vs. PBO-NR sequence), time, and group by time interactions will be included in the MMRM as fixed effects. The study's outcomes will be the change score of ECog, RBANS, and TMT-B from baseline, and the change in NAD+ levels from baseline. We also will examine changes in the FSS, BAI, BDI, and PSQI scores. An unstructured covariance matrix will be used to account for the correlation of repeated measures within a participant. Age, sex, and education will be included in the models as covariates.

9.1.2 Post-Hoc Analysis

The sequential block design will provide the opportunity to examine the effects of NR using a larger sample size, as all participants will ultimately receive 10 weeks of NR (baseline to visit 2 for the NR-NR group; visits 2–4 for the PBO-NR group). In addition to the standard MMRM analysis, we will combine the data from the two randomized groups and explore the pre-post changes in outcomes after 5 and 10 weeks of NR intake compared to baseline. To explore the potential impact of NAD+ levels on change in outcomes, we will perform linear regression analyses of change scores and NAD^+ levels measured after 10 weeks, controlling for age, sex, education, and time since acute COVID-19 infection.

9.1.3 Study Unblinding

After database lock, the responsible statistician will request the treatment codes, the study will be unblinded, and the statistical analysis will be conducted.

10. SAFETY AND ADVERSE EVENTS

10.1 Potential Risks and Benefits

At screening, we will collect standardized historical, symptomatic, physical exam, and laboratory parameters to identify health conditions that represent risks for participation in the study's procedures.

10.1.1 Potential Risks

Nicotinamide Riboside: Nicotinamide riboside is well-tolerated in patients, with few adverse events reported in a minority (3.3%) of patients[16]:

- Mild nausea
- Mild skin rash
- Mild flushing/hot flashes
- Mild leg cramps
- Increased bruising

None of the reported adverse events led to discontinuation of NR during its intervention period.

Phlebotomy: The risks associated with having blood drawn, including the dried blood spot from the finger prick, include bruising and local discomfort. Rarely an infection may occur at this site, and if an infection does occur it will be treated by the study physician.

Magnetic Resonance Imaging: MRI is a safe procedure for subjects who do not have metal implants or other contraindications. Individuals who have electrically, magnetically, or mechanically-activated implants (such as

heart pacemakers) or those who have clips on blood vessels in their brain will not be allowed to participate in the study due to risks associated with MRI scanning. The MRI will be operated in a manner accepted by the FDA. The protocol requires subjects to remain still within a relatively confined space during the scanning session, and the scanner makes loud knocking and beeping sounds as it takes images. While subjects with severe claustrophobia are excluded from the study, some subjects may find the physical confinement or noise uncomfortable. Every effort will be made to enhance each subject's comfort level, and subjects will be given earplugs to reduce discomfort due to noise. If a subject notices any discomfort while in the MRI scanner, (s)he should notify the administrator immediately. If the discomfort cannot be stopped, the scanning session will be stopped. The MRI has the potential, during normal routine use, to cause localized warming of the skin and underlying tissues. Subjects should immediately inform the study staff if they experience discomfort due to warming of the skin and the procedure will be stopped.

Neurocognitive testing: The neurocognitive tests that will be administered to assess mental performance may be stressful and potentially cause anxiety, fatigue, and frustration. In our prior experience with similar protocols, risks have occurred infrequently and very few subjects have terminated testing. However, testing will be discontinued immediately upon any request by the subject to do so. With the evaluation of individuals with cognitive complaints, there is always the potential for a subject to enter the study with subjective cognitive concerns and subsequently demonstrate a decline in cognitive performance throughout the course of the study to a level consistent with a diagnosis of mild cognitive impairment (MCI). If this occurs, a licensed clinician will meet with the subject and suggest that they see their primary care provider for further evaluation.

Questionnaires: Questionnaires administered during the protocol may cause subjects to feel sad or upset about how residual symptoms of COVID-19 are affecting their quality of life. Study staff is experienced with such evaluations and sensitive to these issues. Any question can be omitted per the subject's request.

Wrist Actigraphy: The wrist actigraph, the Fitbit Charge 4, may cause some minor discomfort due to prolonged wearing, but the risk of such discomfort will be minimized by ensuring the wristband fits the subject properly (e.g. is not too tight or does not cause irritation). If the subject does experience discomfort, another wristband will be ordered to try to eliminate the discomfort.

SARS-CoV-2 negative (PCR): The nasal swab that is required for this test may cause some discomfort or, in rare occasions, slight pain.

Videoconferencing: Visits that take place via videoconferencing may allow research staff to learn more about the subject's home and cohabitants than an office visit. The study team will use a secure Zoom videoconferencing platform and conduct the visit in a private location to ensure personal information about the subject is kept confidential.

Other Risks: Reviewing health-related information might be stressful or make the subject feel uncomfortable. Subjects do not have to answer any questions they do not want to. In addition, there may be incidental medical findings as a result of the physical examination. The BDI inquires about suicidality. After the subject completes this instrument during the study visit, the coordinator will review the subjects' answers to these questions. A response of 2 or 3 on Question 9 of the BDI, or any spontaneous expression of suicidality will result in emergent evaluation by a licensed clinician member of study staff for appropriate assessment and triage. If a participant provides a response of "1", it will be considered non-urgent and a licensed clinician member of the

study staff will conduct an appropriate assessment and triage of the participant if necessary either in person, on the phone or virtually via the Zoom platform within 48 hours of being notified.

10.1.2 Known Potential Benefits

NR has been tested in a number of clinical trials in middle-aged and elderly patients and has shown to be well-tolerated. If successful, this trial will further understand of the effects of NR on NAD+ levels, as well as of standard and personalized assessment of cognitive, neuropsychiatric, physical and everyday functioning, in individuals who were infected with COVID-19 and are currently experiencing residual and persisting symptoms. This trial is also assessing multiple biomarkers in concert with clinical outcomes, which will provide a detailed understanding of NR activity and provide a well-curated data set for the community of individuals who had COVID-19 to improve our understanding of the long-term effects of COVID-19.

10.2 Data Security

10.2.1 Laptop Computer

Subjects will be provided a study-laptop during the visit to complete self-reported measures (i.e., BDI, BAI, PCL-C, ECog, BRIEF-A, PSQI, IPAQ, Fatigue Severity Scale) on REDCap and the online Continuous Performance Test (CPT). The laptop will be used only to complete the questionnaires on REDCap and the CPT. A study coordinator will always be present to address questions. No PHI or personally identifiable information will be entered into the system, so there are no additional data safety risks to the subject. The study laptop will be registered under the Principal Investigator and no identifying information will be required to sign in. There will be a password that will be available to the Principal Investigator and study coordinators, and not the participants. The computer has been encrypted using Bitlocker, per MGB's policies.

10.2.2 Mobile device

Fitbit is a web-based application that require no PHI or personally identifiable information be entered into the system, so there are no additional data safety risks to the subject if they use their personal mobile device, tablet or computers. Subjects will sync Fitbit activity tracking through the Fitbit app downloaded to their personal mobile device, tablet or computers by logging into the application with their provided, deidentified account.

10.2.3 Fitbit activity tracker

A study coordinator will create a unique, deidentified email and password combination to create an account on Fitbit.com. To create an account, the following information will be required: first and last Name (a de-identified placeholder name], date of birth (January 01, year of birth), gender, height, and weight. The subject will be given the associated account information so that they are able to login, sync their device, and use the Fitbit dashboard. The subject will be notified that the researcher will also have full access to their account and will be able to freely view and download the subject's data for research purposes. A copy of Fitbit's Privacy Policy and Terms of Service will also be reviewed by the subject during the consenting process. By consenting to the study, the subject is also agreeing to these policies and terms.[41]

After the subject has completed or if they are withdrawn from the study, their Fitbit account and all associated data will be deleted. However, prior to deleting the account, the de-identified study data will be downloaded and stored confidentially on Partner's computers for study purposes.

11. MONITORING AND QUALITY ASSURANCE

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonisation (ICH) guidelines. The PI will carefully monitor each subject throughout the study for possible adverse events. All AEs will be documented on CRFs designed specifically for this purpose. It will be important to report all AEs, whether serious or non-serious.

11.1 Independent Monitoring of Source Data

The PI will ultimately be responsible for the validity and integrity of the data collected at the MGH site, and for ensuring that the study is conducted in accordance with the IRB-approved protocol. After data is collected and recorded on forms, the study coordinator may input the data into the REDCap database. Entries will be reviewed for accuracy and completeness by a second study coordinator. Study coordinators will also review the subjects' responses on REDcap to ensure that no data is missing. Finally, the PI or their designee (Co-I) will conduct monthly reviews to check that data in REDCap accurately reflects the data collected on the original data capture forms. The research team (PI, Co-I, research coordinators) will subsequently meet to discuss the results of this review, as well as case report forms and source documentation.

All electronic documentation will be stored on password-protected devices in locked cabinets located in secured areas. Paper forms will be stored in locked cabinets located in secured areas.

11.2 Safety Monitoring

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The PI will carefully monitor each subject throughout the study for possible adverse events. All AEs will be documented on CRFs designed specifically for this purpose. It will be important to report all AEs, whether serious or non-serious.

11.3 Definitions of AEs & SAEs

11.3.1 Adverse Event

An adverse event (AE) is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device, whether or not considered related to the drug product or device.

Examples of adverse events include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (i.e. skin rash, peripheral edema, etc.), or clinically significant abnormal test results (i.e. lab values or vital signs), with the exception of outcome measure results, which are not being recorded as adverse events in this trial (they are being collected, but analyzed separately). Stable chronic conditions (e.g. arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered adverse events. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity are considered as worsened and therefore would be recorded as adverse events. Adverse events are generally detected in two ways:

- Clinical → symptoms reported by the subject or signs detected on examination.
- Ancillary Tests → abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures, the results of which are not being captured as AEs).

If discernible at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the PI and recorded on the AE log. However, if an observed or

reported sign, symptom, or clinically significant laboratory anomaly is not considered by the PI to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the AE log. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the PI.

Subjects will be monitored for AEs from the time they sign consent until completion of their participation in the study (defined as death, consent withdrawal, loss to follow up, early study termination for other reasons, or following completion of the entire study). An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current Investigator's Brochure.

The study procedures and the well-being of all participants will be monitored closely by the MGH principal investigator and the Co-Investigators. Throughout the course of the study, constant feedback with the subject is maintained in order to assess comfort and safety and to minimize risks throughout the procedure. The above investigators will be responsible for determining if a subject should be removed from the study. Criteria for removal include the following: 1) if a subject is unwilling or unable to participate in study procedures 2) if the subject refuses to participate and consent, 3) if the subject acquires a medical condition that prohibits further participation, 4) if in the opinion of the MGH principal investigator, Dr. Edmarie Guzmán-Vélez, it is decided that it is not in the subject's best interest to continue participation.

Unanticipated problems including adverse events will be reported to the Partners Human Research Committee (PHRC) as described in the PHRC policy on Unanticipated Problems Involving Risks to Subjects or Others including adverse events.

The Data Management team will be responsible for the development, execution, and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets. All data will be managed in compliance with applicable regulatory requirements. The study coordinator, under the supervision of the PI, will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and/or other forms used to report, track, and record clinical research data. The study coordinator will be instructed to enter this information into the REDCap database. The REDCap platform provides password protection. An edit checking and data clarification process will be put in place to ensure accuracy of the data. Logic and range checks as well as more sophisticated rules may be built into the eCRFs to provide immediate error checking of the data entered. The system has the capability to automatically create electronic queries for forms that contain data that are out of range, out of window, missing, or not calculated correctly. The platform will have the ability to lock specific visits to prevent any modification of data once the visit is closed. Once this option is activated, every user will have Read-Only access to the data. The PI, Dr. Edmarie Guzmán-Vélez, will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

11.3.2 Serious Adverse Events

All adverse events will be reviewed by the MGH Principal Investigator, Dr. Edmarie Guzmán-Vélez, and will be reported to Partners IRB and to the Human Research Committee (HRC) in accordance with HRC Guidelines. A serious adverse event (SAE) is defined as an adverse event that meets any of the following criteria:

1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurs.
    - This serious criterion applies if the study subject, in the view of the PI, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.

- Hospitalization for an elective procedure or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled “procedure” or a “treatment” is not an untoward medical occurrence.

4. Results in persistent or significant disability or incapacity.
   - This serious criterion applies if the “disability” caused by the reported AE results in a substantial disruption of the subject’s ability to carry out normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject.
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical adverse event may meet criteria for "seriousness" but is not an adverse experience and will therefore not be considered an SAE. An example of this would include a social admission (subject admitted for reasons other than medical, e.g., lives far from the hospital, has no place to sleep).

The PI is responsible for classifying adverse events as serious or non-serious.

11.4 Assessment and Recording of Adverse Events

The PI will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. All AEs will be collected and reported in the EDC system.

11.4.1 Assessment of Adverse Events

At each visit (including telephone visits), the subject will be asked if they had any problems or symptoms since their last visit in order to determine the occurrence of adverse events. If the subject reports an adverse event, the Investigator will probe further to determine:

- Type of event
- Date of onset and resolution (duration)
- Severity (mild, moderate, severe)
- Seriousness (does the event meet the above definition for an SAE)
- Causality, relation to investigational protocol
- Outcome

11.4.2 Relatedness of Adverse Event to Investigational Protocol

1. Not Related:	Concomitant illness, accident, or event with no reasonable association with protocol.
2. Unlikely:	The reaction has little or no temporal sequence from administration of the investigational protocol, and/or a more likely alternative etiology exists.
3. Possibly Related:	The reaction follows a reasonably temporal sequence from administration of the investigational protocol and follows a known response pattern to the suspected investigational protocol; the reaction could have been produced by the investigational protocol or could have been produced by the subject’s clinical state or by other modes of therapy administered to the subject.

4. Probably Related:	The reaction follows a reasonably temporal sequence from administration of investigational protocol; is confirmed by discontinuation of the investigational protocol or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state.
5. Definitely Related:	The reaction follows a reasonable temporal sequence from administration of investigational protocol; that follows a known or expected response pattern to the investigational protocol; and that is confirmed by improvement on stopping of the investigational protocol, and reappearance of the reaction on repeated exposure.

11.4.3 Recording of Adverse Events

All clinical adverse events are recorded in the Adverse Event (AE) Log in the subject's study binder. Study staff should fill out the AE Log and enter the AE information into the EDC system within 48 hours of learning of a new AE or receiving an update on an existing AE.

Entries on the AE Log (and into the EDC) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event.

11.5 Adverse Events and Serious Adverse Events - Reportable Events

The following are considered reportable events and must be reported via the EDC system within 24 hours of study staff being notified of the event.

- All events that meet the above criteria for Serious Adverse Events

11.6 Safety and Feasibility of Performing Experimental Visits at MGH-East (Charlestown Navy Yard)

The entire study will be performed in MGH Building 149 in examination, testing and procedure rooms of the Neurology Service on the 2nd floor. If an emergency does occur, 911 will be called promptly, with the subject being managed by the study physician until paramedics arrive. This setup has been discussed with and approved by the nursing administration at the CNY Clinical Research Center.

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