Efficacy and safety of N-acetylcysteine (NAC) in patients with mild vascular cognitive impairment

A randomized, double-blind, placebo-controlled parallel group design study

Version 1.9

NCT03306979

Principal Investigator:	*Dr. Krista L. Lanctôt, PHD*
Qualified Investigator:	*Dr. Damien Gallagher, MD FRCP(C)*
Regulatory Sponsor:	Sunnybrook Research Institute
Funding Agency:	Canadian Institutes of Health Research
Investigational Product:	*N-acetylcysteine (NAC)*

SPONSOR STATEMENT OF COMPLIANCE

This study will comply with the International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Participants, as well as applicable regulatory and institutional requirements.

Name: *(Print)*	*Dr. Krista L. Lanctôt*
Title: *(Print)*	*Principal Investigator*
Signature:	
Date of Approval: *(yyyy-mmm-dd)*	

Name: *(Print)*	*Dr. Damien Gallagher*
Title: *(Print)*	Qualified Investigator
Signature:	
Date of Approval: *(yyyy-mmm-dd)*	

PROTOCOL SIGNATURE PAGE

I have read this protocol in its entirety I and its appendices. I agree to comply with the requirements of the study protocol and procedures for data recording/reporting and acknowledge my responsibility for the well-being of each research participant, and to ensure that all persons involved in study activities are adequately informed about the protocol, the investigational product, and their trial-related duties. The signature below constitutes the agreement to conduct this study in accordance with the REB approved protocol, GCP and applicable regulatory requirements, including confidentiality, ethical guidelines and regulations regarding the conduct of research in humans.

Qualified Investigator:

Name & Title: *(Print)*	Dr. Damien Gallagher, MD FRCP (C)
Institution: *(Print)*	Sunnybrook Health Sciences Centre
Signature:	
Date of signature: *(yyyy-mmm-dd)*	

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

The following abbreviations describe terms, documents and study personnel used in the conduct of this study protocol.

AD	Alzheimer's disease
AE	Adverse Event/Adverse Experience
ARWMC	Age-Related White Matter Change
CAD	Coronary Artery Disease
ccf-mtDNA	Circulating cell-free mitochondrial DNA
CR	Cardiac Rehabilitation
CRF	Case Report Form
CTS	Clinical Trial Services
ICH	International Conference on Harmonization
IP	Investigational Product
pCRF	Paper Case Report Form
GCP	Good Clinical Practice
GSH	Glutathione
ICF	Informed Consent Form
MCI	Mild Cognitive Impairment
NAC	N-acetylcysteine
NINDS-CSN	National Institute of Neurological Disorders and Stroke-Canadian Stroke Network
OS	Oxidative Stress
PHI	Personal Health Information
PI	Principal Investigator
PlGF	Placental Growth Factor
PM	Product Monograph
QI	Qualified Investigator
REB	Research Ethics Board
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
SRI	Sunnybrook Research Institute
SUADR	Serious and Unexpected Adverse Drug Reaction
VCI	Vascular Cognitive Impairment

PROTOCOL SUMMARY

Protocol Title (Short Title)	Efficacy and Safety of N-acetylcysteine in patients with mild vascular cognitive impairment (MOVE-IT)
Protocol Number	*1.9*
Phase	II
Study Design	Randomized, double-blind, placebo-controlled parallel group
Study Duration	5 years
Setting	Single-centre
Sample Size	60
Main Inclusion Criteria	Males or females aged 55-85 years, with mild vascular cognitive impairment (VCI), who are enrolled in a supervised cardiac rehabilitation program.
Primary Outcome(s):	In patients with mild VCI, those randomized to NAC (2,400 mg/day) will demonstrate greater improvement in executive function over 24 weeks as compared to those randomized to placebo.
Secondary Outcome(s):	In patients with mild VCI, those randomized to NAC (2,400 mg/day) will demonstrate greater improvement in processing speed, memory and working memory over 24 weeks as compared to those randomized to placebo.
Investigational Product and Planned Use	N-acetylcysteine (NAC) Natural Health Products Directorate (NPN #: 80004844). Patients will be randomized to receive NAC (four 600 mg capsules given as 2 capsules in the morning and 2 capsules in the evening) or matching placebo capsules. The initial NAC dosage will be 600mg/day (one 600mg capsule in the morning) for the first week, followed by 1,200 mg/day (one 600 mg capsule in the morning, one 600mg capsule in the evening) for the second week, followed by 1,800 mg/day (two 600mg capsules in the morning, one 600mg capsule in the evening) for third week, followed by 2,400mg/day (two 600mg capsules in the morning, two 600mg capsules in the evening) for the following 21 weeks.
Statistical Analysis:	Differences in executive function composite z scores between the groups at 24 weeks will be compared between groups using a two sample two sided t-test. A linear mixed model will be run to compare the rate of change in the executive function measure over time.

1 KEY ROLES AND CONTACT INFORMATION

Regulatory Sponsor:	Sunnybrook Research Institute (SRI) 2075 Bayview Avenue Toronto, ON Canada M4N 3M5
Principal Investigator:	Dr. Krista L. Lanctôt, PHD Sunnybrook Health Sciences Centre 2075 Bayview Avenue Toronto, Ontario M4N 3M5 Tel: (416) 480-6100 x2241
Academic Research Organization:	Clinical Trial Services, SRI 2075 Bayview Avenue Toronto, ON Canada M4N 3M5
Qualified Investigator:	Damien Gallagher, MD FRCP(C) Sunnybrook Health Sciences Centre 2075 Bayview Avenue Toronto, Ontario M4N 3M5 Tel: (416) 480-4098

2 INTRODUCTION

This study document is the protocol for research involving human participants. This study is to be conducted according to Canadian and international standards, and in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP), World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Participants, as well as applicable regulatory and institutional requirements and research policies.

2.1 Background and Rationale

2.1.1 Vascular cognitive impairment-no dementia

The prodromal stage of major neurocognitive disorders has become the current target for interventional research, as this stage may provide an optimal therapeutic window for neuroprotection. Mild vascular cognitive impairment is a prodromal stage defined by early cognitive deficits due to cerebrovascular disease that are not severe enough to be classified as dementia and is the most prevalent form of vascular cognitive impairment **(VCI)** among those aged 64 to 84 years[1]. Probable mild VCI is characterized by neuroimaging findings confirming vascular pathology; clinical symptoms indicating vascular disease in the absence of these neuroimaging findings is categorized as possible mild VCI[2].

Consistent with the recognition of vascular contribution to cognitive impairment and dementia[3], those with **coronary artery disease (CAD)** are particularly at risk for mild VCI. Clinical trials evaluating the efficacy of cholinesterase inhibitors and memantine, as well as primary prevention trials to control traditional vascular risk factors have demonstrated no significant benefit of these interventions in the prodromal stage[4-6]. Regular exercise is promising[7,8], but has variable efficacy on cognition[9-11], particularly in the preclinical stages[11]. There is currently no effective treatment for those at increased risk for dementia[4].

2.1.2 Oxidative stress

Oxidative stress **(OS)** plays a major role in age-related neurodegeneration[12]. Cumulative reactive oxygen species cause cellular damage, mitochondrial dysfunction and impair the DNA repair system, all of which are key factors involved in the propagation of neuronal injury leading to apoptosis. Consistent with this, increased oxidative damages have been implicated as early events in the evolution of the neurodegenerative processes[13,14]. Similarly, decreased levels of glutathione **(GSH),** the primary antioxidant in the brain, are observed in patients with neuropsychiatric disorders [15]. Antioxidant supplementation has been proposed for dementia prevention and therapy[16-18]. Observational and epidemiological studies with dietary or supplemented antioxidants showed benefits[18,19], although findings from controlled trials have been mixed[20]. Of those studies, therapy with the antioxidant N-acetylcysteine **(NAC)** showed promise, consistent with action as an effective GSH precursor[21].

Importantly, emerging evidence suggests that altered OS might represent an early event in the neurodegenerative process. A recent meta-analysis showed that lipid peroxidation was significantly elevated while a range of antioxidant levels were depleted in mild cognitive impairment **(MCI)** patients [22-28]. OS may be particularly important in mild VCI as cerebral

hypoperfusion can cause ischemia- and inflammation- related increases in OS[29]. Our pilot data showed that high OS predicted less cognitive improvement following a 6-month exercise intervention, suggesting that elevated OS levels might interfere with the benefit of exercise on cognition.

2.1.3 The therapeutic potential of antioxidant supplementation N-acetylcysteine for cognition

GSH depletion plays an important role in OS-mediated neuronal loss[13]. As such, increasing central GSH has been proposed as a promising therapeutic strategy in Alzheimer's disease **(AD)**[21]**.** NAC protects the cells against OS damage by increasing the endogenous intracellular GSH level. NAC bioavailability, metabolism and plasma levels have been studied in humans[30-32]. NAC has been shown to cross the blood brain barrier and accumulate in the central nervous system[30,33,34], where it lowers lipid peroxidation[35] and beta-amyloid deposition[36]. NAC also protects neuronal function and improves cognitive deficits by decreasing acetylcholinesterase activity and increasing acetylcholine levels[37]. Clinical studies in patients with AD suggest that NAC may have pro-cognitive effects. Patients with mild to moderate AD treated with NAC for 24 weeks showed significant improvement in performance on the Letter Fluency Task and Wechsler Memory Scale Immediate Number Recall[38]. In a 12-month small open label trial, a vitamin/nutraceutical formulation containing NAC produced a significant effect on executive function in individuals with early stage AD and MCI[39-41]. However, to date, no studies have evaluated the effects of NAC on cognition in patients with mild VCI.

Those with mild VCI show deficits across all cognitive domains, with greater impairment typically being found in executive function. Impairment in cognitive subdomains of memory, processing speed and working memory have also been found in mild VCI populations[42]. Our recent pilot data showed CAD patients with possible mild VCI had elevated levels of OS compared to CAD controls. In addition, higher baseline ratios of late to early lipid peroxidation markers (8-isoprostane (8-ISO) to lipid hydroperoxides (LPH) ratio) predicted less improvement in executive function following an exercise intervention. Since OS damage occurs early in the pathogenesis of the disease and might precede other neuropathological alterations[23,43], an antioxidant intervention would be most beneficial if started before the onset of symptomatic dementia. We propose to combine the synergistic effects of NAC supplementation and exercise to increase the efficacy of each individual intervention to reduce OS and improve cognitive performance in patients with mild VCI. [44,45]. If successful, this study will demonstrate that NAC, a widely available and safe supplement, can improve cognitive outcomes in patients at high risk of cognitive and functional decline.

3 STUDY OBJECTIVES

3.1 Primary Objective

As highlighted above, NAC preferentially improved executive function. Our primary goal is to evaluate the therapeutic potential of direct antioxidant supplementation using **NAC** to enhance the effect of exercise on executive function in patients with mild VCI. We hypothesize that:

- In patients with mild VCI, those randomized to NAC (2,400 mg/day) will demonstrate greater improvement in executive function over 24 weeks as compared to those on placebo.

Executive function will be based on verbal fluency and trail test A & B found in the 60-minute battery recommended by the National Institute of Neurological Disorders and Stroke-Canadian Stroke Network (**NINDS-CSN**) harmonized standards. It is the cognitive domain most frequently impacted in mild VCI[42,46], and it reflects white matter lesion burden, the key neuroimaging finding defining probable mild VCI. The 24 week treatment duration was chosen based on a previous clinical trial of NAC in patients with probable AD[38]. The daily dose is consistent with the upper dosing range in published clinical trials, in which oral NAC supplementation was investigated in a variety of populations including AD, where evidence of tolerability and efficacy were observed[34,38,47-50].

3.2 Secondary Objective(s)

Individuals with mild VCI also show deficits in processing speed, memory and working memory[42]. Our secondary goal is to evaluate the therapeutic potential of NAC to enhance the effect of exercise on additional cognitive domains in these patients. We hypothesize that:

- In patients with mild VCI, those randomized to NAC (2,400 mg/day) will demonstrate greater improvement in processing speed over 24 weeks as compared to placebo.
- In patients with mild VCI, those randomized to NAC (2,400 mg/day) will demonstrate greater improvement in memory over 24 weeks as compared to placebo.
- In patients with mild VCI, those randomized to NAC (2,400 mg/day) will demonstrate greater improvement in working memory over 24 weeks as compared to placebo.

Processing speed will be based on the Symbol Digit Modalities Test and the memory domain, will consist of the Rey Complex Figure Test-immediate/delayed recall and recognition and Hopkins Verbal Learning Test (HVLT) -immediate/delayed recall and recognition, found in the 60-minute battery recommended by the NINDS-CSN. Working Memory will be based on Digit Span test (Forward, Backward, and Sequencing subtests).

NAC crosses the blood brain barrier and acutely increases blood and brain GSH concentrations[51]. To demonstrate target engagement, we aim to evaluate the effect of NAC on the GSH antioxidant system and OS. The antioxidant levels will be evaluated by measuring levels of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferases (GST)[15]. OS will be quantified by measuring concentrations of late markers (e.g., 8-ISO and 4-HNE) and early marker (LPH) lipid peroxidation products[52]. LPH is one of the primary and earliest products of lipid peroxidation, which are inherently unstable and susceptible to further free radical-mediated

reactions, which results in the generation of late-stage oxidative stress products. Higher OS will be indicated by higher ratio between late marker (e.g., 8-ISO) and early marker (LPH) of lipid peroxidation as supported by our pilot data.

- In patients with mild VCI, the plasma levels of GPx, GR and GST will be significantly higher in those receiving NAC supplementation relative to those who received exercise intervention alone.
- In patients with mild VCI, the 8-ISO to LPH ratio will be significantly lower in those receiving NAC supplementation relative to those who received exercise intervention alone

Evidence from neuroimaging studies in VCI patients indicated greater severity and progression of white matter lesion burden was associated with greater declines in executive function and processing speed[53-55]. We will investigate whether baseline WMH severity significantly predicts the cognitive response to NAC.

- In patients with mild VCI, those with lower WMH volume at baseline will demonstrate greater cognitive improvement in response to 24 weeks of NAC treatment

Previous trials have shown that NAC at these doses is well-tolerated in the elderly[38,56,57]. We will investigate tolerability of NAC.

- In patients with mild VCI, measures of safety (including incidences of adverse events and drop-outs) will be comparable between treatment with NAC and placebo.

3.3 Exploratory Hypotheses

The antioxidant defense molecule GSH serves as the primary antioxidant in the brain by scavenging harmful reactive oxygen species generated during cellular metabolism. GSH depletion plays an important role in OS-mediated neuronal death[53], precedes neurodegeneration[54], and contributes to the etiology of various neurodegenerative diseases[55]. Advances in magnetic resonance spectroscopy (MRS) now allow in vivo non-invasive quantification of GSH in the brain. We propose an innovative methodology to investigate the association between brain GSH and cognitive performance in subjects with mild VCI who are likely to have higher oxidative stress and GSH depletion.

- In these patients, those with higher GSH levels at baseline will demonstrate greater improvement in executive function over 24 weeks.
- In these patients, those with higher GSH levels at baseline will demonstrate greater improvement in processing speed over 24 weeks.
- In these patients, those with higher GSH levels at baseline will demonstrate greater improvement in memory over 24 weeks.
- In these patients, those with higher GSH levels at baseline will demonstrate greater improvement in working memory over 24 weeks.

Apathy is a prominent neuro-behavioral symptom of vascular dementia;[58] it is defined as an impairment in motivation that persists over time. Unfortunately, apathy has rarely been assessed in mild VCI. We propose to assess apathy in these patients using the Apathy Evaluation Scale (AES) and the Mild Behavioral Impairment Checklist (MBI-C).[59,60] We will explore the possible associations between apathy, blood biomarkers, and the therapeutic potential of NAC in these patients.

White matter lesions is the key neuroimaging finding defining probable mild VCI[2]. We will define those with clinical symptoms indicating vascular disease, and negative neuroimaging findings as possible mild VCI. We hypothesize that patients with probable mild VCI will have higher OS than patients with possible mild VCI. We will explore baseline brain GSH levels in patients with probable mild VCI compared to patients with possible mild VCI. Where possible, we will also test our primary and secondary outcomes in the probable mild VCI subgroup.

Oxylipins are lipid species formed from the oxygenation of polyunsaturated fatty acids; they play a role in inflammation and vascular function[61,62]. Oxylipins will be explored in association with cognitive and mood symptoms. Mitochondrial dysfunction has been linked to inflammation and several diseases including Alzheimer's disease and cardiovascular disease. Mitochondrial dysfunction will be measured by circulating cell-free mitochondrial DNA (**ccf-mtDNA**). We will explore its relationship with blood biomarkers, cognition, and neuroimaging biomarkers. Placental growth factor (**PlGF**) is a protein belonging to the vascular endothelial growth factor family and higher levels are associated with CAD[63]. It is proposed to stimulate angiogenesis as a response to pathological events, such as cardiovascular disease and ischemia[64], and can be enhanced through exercise [65]. We will explore the clinical relevance of PlGF, its relationship with neuroimaging biomarkers, and its relationship with cognition. Astrocyte performs a crucial role in central GSH synthesis and homeostasis. Astrocyte depletion may be a biomarker of OS mediated astrocyte dysfunction that precipitates change in brain GSH. Astrocytic damage and dysfunction will be quantified by measuring the S100 calcium-binding protein B (S100B). S100B is a biomarker secreted by astrocytes that enters the bloodstream[66]. Evidence shows that S100B was consistently elevated in the brain, cerebrospinal fluid and serum among mild/moderate AD[67-69]. We will explore possible associations between S100B and brain GSH levels.

Recent studies suggest a role of innate immune activity, including the effects of neutrophils, in neurodegeneration and cognitive decline[70,71]. Myeloperoxidase (MPO), an enzyme primarily found in the auzophilic granules of neutrophils, catalyses the formation of reactive oxidant species (ROS)[72]. Previous research has reported higher blood concentrations of MPO in CAD patients[73] and serum MPO concentration has been associated with future risk of developing CAD[74]. High concentration of peripheral MPO have also been reported in individuals with vascular dysfunction, such as high WMH burden[75] and AD[76]. We will explore possible associations between MPO and specific cognitive domains. Examining this relationship will enable us to identify upstream contributors of inflammation and OS associated with symptoms of mVCI.

4 METHODS

4.1 Study Design

This is a 24-week randomized, double-blind, placebo-controlled parallel group design study involving mild VCI patients to evaluate the efficacy and safety of oral NAC supplementation (2,400 mg daily) as an add-on therapy to improve cognitive function in patients undergoing cardiac rehabilitation **(CR)**. The CR program consists of a harmonized aerobic and resistance training in a supervised group setting. Eligible patients will be randomized to receive NAC (four 600 mg capsules given as 2 capsules in the morning and 2 capsules in the evening) or matching placebo capsules. The initial NAC dosage will be 600mg/day (one 600mg capsule in the morning) for the first week, followed by 1,200 mg/day (one 600 mg capsule in the morning, one 600mg capsule in the evening) for the second week, followed by 1,800 mg/day (two 600mg capsules in the morning, one 600mg capsule in the evening) for third week, followed by 2,400mg/day (two 600mg capsules in the morning, two 600mg capsules in the evening) for the following 21 weeks.

4.2 Subjects

This study will be approved by the Research Ethics Board of Sunnybrook Health Sciences Centre (Sunnybrook) and the University Health Network (UHN). All patients will provide written, informed consent.

Mild VCI will be diagnosed by a clinical history of vascular disease, the presence of mild cognitive impairment as defined by modest deficits (1 SD below population norm) in executive function, memory, processing speed or working memory. Subjects will be categorized as "possible mild VCI", those that meet neuroimaging criteria (below) will be categorized as "probable mild VCI".

Probable mild VCI will be diagnosed using the core diagnostic criteria for Subcortical Ischemic Mild Cognitive Impairment[3,77], which include (A) Presence of mild cognitive impairment, as defined by modest deficits (1 SD below population norm) in executive function, memory, processing speed or working memory; (B) Absence of prior symptomatic stroke (silent brain infarcts are allowed); (C) Presence of "diffuse, subcortical cerebrovascular disease", defined as 1) the presence of two or more silent brain infarcts in supratentorial locations or 2) extensive white matter disease (Age-Related White Matter Change [**ARWMC**] scale score[78] of 2 or higher, indicating "beginning confluence of lesions" or greater) in any brain region.

5 PARTICIPANT SELECTION AND WITHDRAWAL

A total of 60 mild VCI patients will be recruited from the CR program at the University Health Network Toronto Rehabilitation Institute.

5.1 Inclusion Criteria

Each mild VCI participant must meet all of the following inclusion criteria at baseline:

- Males or females aged 55-85 years.
- MoCA score indicating cognitive impairment (less than 28 out of 30; Telephone MoCA: less than 20 out of 22)
- Modest deficits (1 SD below population norm) in executive function, memory, processing speed or working memory based on the 60-minute battery recommended by the NINDS-CSN.
- Speaks and understands English.
- Enrollment in the Cardiac Rehabilitation program at the University Health Network Toronto Rehabilitation Institute.

In addition, to meet criteria for **probable mild VCI**

- Presence of "diffuse, subcortical cerebrovascular disease", defined as 1) the presence of two or more silent brain infarcts in supratentorial locations or 2) extensive white matter disease (Age-Related White Matter Change [**ARWMC**] scale score[78] of 2 or higher, indicating "beginning confluence of lesions" or greater) in any brain region.

Participants who exhibit modest deficits (1 SD below population norm) in executive function, memory, processing speed or working memory based on the 60-minute battery recommended by the NINDS-CSN but do not have MRI findings of "diffuse, subcortical cerebrovascular disease" will be categorized as **possible mild VCI**.

5.2 Exclusion Criteria

All participants meeting any of the following exclusion criteria at baseline will be excluded from participation in this study:

- A history of stroke
- A history of epilepsy
- Uncontrolled asthma (requiring hospitalization or ER visit in the last 3 months by patient report)
- Uncontrolled diabetes (clinical determination)
- Severe hypo/hypertension (clinical determination)
- Uncontrolled hypercholesterolemia (clinical determination)
- Presence of significant medical illnesses:
    - ❑ Severely disturbed liver function
    - ❑ Severely disturbed kidney function
    - ❑ Severely disturbed lung function
    - ❑ HIV, HBV and/or HCV infection
    - ❑ Malignant tumors

- A current neurological condition
  - ❑ Parkinson's disease
  - ❑ Multiple sclerosis
  - ❑ Significant traumatic brain injury
- Major psychiatric condition
  - ❑ Current major depressive disorder
  - ❑ Schizophrenia
  - ❑ Bipolar disorder
  - ❑ Substance use disorder (alcohol abuse, heavy smoking (20 cigarettes or more/day))
- Contraindication to MRI or MRS (e.g. metal in body, pacemaker).
- Contraindication to NAC (documented allergy) or allergy to lactose.
- Daily nitroglycerin use.
- Bleeding disorders (e.g. hemophilia, thrombotic thrombocytopenic purpura) and/or elective surgery within 30 days.
- Volunteers who currently participate in another pharmacological study.

5.3 Participant Recruitment

Participants will be recruited for the study from the cardiac rehabilitation program at Toronto Rehabilitation Institute. Study personnel will make initial contact with potential subjects and will explain all the details of the study. Participants will be given as much time as they need to review the information sheet and consent form in order to make an informed decision to consent to participate in the study.

5.4 Participant Withdrawal and Discontinuation of IP

5.4.1 Reasons for Withdrawal/Discontinuation of IP

At their own discretion, participants may withdraw from the study at any time and for any reason. Study participants will be withdrawn from the study if consent is withdrawn, and will be considered for study withdrawal for non-compliance, significant protocol violation, NAC intolerance and/or severe adverse events, the development of new medical conditions which prevent the continuation of participation, being uncooperative during the study, or if continuing in the study is in conflict with the best interest of the patient. Any patients with emergent AEs will be followed until the Aes are resolved.

5.4.2 Data Collection and Follow-up for Withdrawn Participants

Participants who withdraw from the study will be contacted by the study research team to request a final visit and to follow up any unresolved adverse events. After the early termination visit, no further study procedures or evaluations will be performed, or additional study data collected. Any data collected prior to the withdrawal of consent may be retained and used by the sponsor.

6 INTERVENTIONS

6.1 Investigational Product

N-acetylcysteine (NAC), Natural Health Products Directorate (NPN #: 80004844), supplementation will be used as an add-on therapy to improve cognitive function in patients undergoing cardiac rehabilitation.

6.1.1 Intervention

Patients will be randomized to receive NAC (four 600 mg capsules given as 2 capsules in the morning and 2 capsules in the evening) or matching placebo capsules. The initial NAC dosage will be 600mg/day (one 600mg capsule in the morning) for the first week, followed by 1,200 mg/day (one 600 mg capsule in the morning, one 600mg capsule in the evening) for the second week, followed by 1,800 mg/day (two 600mg capsules in the morning, one 600mg capsule in the evening) for third week, followed by 2,400mg/day (two 600mg capsules in the morning, two 600mg capsules in the evening) for the following 21 weeks. The dose will be escalated (to 2,400mg), unless there are tolerability issues (assessed by QI), in which case the QI will decide which dose is appropriate for the patient.

The Pharmacy Department at Sunnybrook Health Sciences Centre will prepare, label and dispense the experimental and placebo (lactose-filled) capsules. The Pharmacy Department at Sunnybrook Health Sciences Centre will prepare the placebo capsules by using empty capsule shells supplied by SISU, a Canadian manufacturer, and filling them with a lactose-based filler. To make experimental and placebo capsules indistinguishable with regard to taste and appearance, a process will be implemented to mimic the smell of NAC, as done previously[47]. The investigational product and placebo capsules will be stored in a secure location at Sunnybrook in a temperature monitored area. Placebo and intervention capsules will be provided by SISU, and licensed in accordance with Natural Health Products Regulations. Details regarding this preparation will be filed in the Natural Health Products Directorate (NPN #: 80004844). The Pharmacy Department at Sunnybrook Health Sciences Centre will destroy the experimental and placebo capsules.

6.1.2 Randomization and Masking

A block randomization code will be independently computer-generated by the Clinical Trial Services at SHSC and remain locked in a secure location in that department. Kits with study medication will be consecutively pre-packaged as per randomization sequence prior to commencement of the trial. All study personnel will remain blind to treatment allocation and block size until the final patient has completed follow-up and the database is locked. Unblinding will not be allowed unless there exists exceptional clinical circumstances that justify it, such as if acute medical management of an SAE is required. Individuals performing study related measures (e.g., plasma analyses, imaging analyses) will also remain blind to treatment allocation and all clinical data throughout the entire duration of the study.

6.1.3 Receiving and Storage

6.1.3.1 Receipt of Investigational Product

SISU will ship the investigational product to the Pharmacy Department at SHSC and will provide the qualified investigator with chain of custody documentation as well as certificates of analysis.

Upon receipt of the investigational product, the Pharmacy will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable product in a given shipment will be documented in the study files and study staff will be informed.

SISU will ship the empty capsule shells to the Pharmacy Department at SHSC and will provide the qualified investigator with chain of custody documentation as well as certificates of analysis. Upon receipt of the empty capsule shells, the Pharmacy will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable capsules will be documented in the study files and study staff will be informed.

6.1.3.2 Storage and Stability

The investigational product will be stored in a secure location in a temperature monitored area at Sunnybrook Health Sciences Centre. There are no special handling requirements for storing NAC.

6.1.4 Participant Compliance Monitoring

Compliance will be monitored using capsule counts (non-compliance is defined as taking < 80% of their capsules). Study participants will not be withdrawn from the study for non-compliance unless the PI/QI have safety concerns.

7 STUDY SCHEDULE AND PROCEDURES

7.1 Assessments

A schedule of assessments is provided in Appendix A. Demographic data, including age, gender, BMI, level of education, vascular risk factors (hypertension, smoking, hypercholesterolemia, obesity, and diabetes), concomitant medications, and language and medical history will be collected during the first screening visit. The MRI scan will be done at the second screening visit. Individuals that cannot have an MRI can still be enrolled in the study; they will be classified as possible mVCI. The effect of exercise will be operationalized using the VO_2 peak. GST, S100B and lipid peroxidation markers, and other blood-based biomarkers will be assessed as stated in section 7.3. APOE4 non-carriers might be more susceptible to oxidative stress and thus more likely to benefit from antioxidant treatment[17,79]. Blood samples will be collected, to determine APOE4 genotype. Dietary intake (including antioxidant supplementation), vital signs, and safety outcomes will be evaluated as shown on Appendix A.

Cognitive performance will be assessed using the 60-minute battery recommended by the NINDS-CSN harmonized standards[80]. The NINDS-CSN was chosen based on its ability to characterize individuals with VCI, particularly in the early stages[80]. In a recent study, the 60-min protocol was shown to effectively discriminate between cognitively intact individuals and those with cognitive impairment of varying degrees, particularly in patients with cognitive impairment-no dementia (AUC range: 0.80- 0.90)[46]. Additionally, the Digit Span Test (Forward, Backward and Sequence) will be administered as part of the cognitive battery. General cognitive function will be assessed using the MoCA, which has superior sensitivity to detect cognitive deficits for mild VCI[81]. Apathy will be assessed using the AES and MBI-C.[59,60] For each cognitive task, a Z-score will be determined based on published age- and sex- matched norms. The Z distribution maps the population mean of the test variable to 0, with better performance being more positive and poorer performance being more negative based on a normal distribution. The composite z-scores for each cognitive domain will be calculated by summing the Z-scores of individual test scores within the particular domain.

7.2 MRI SCAN

The MRI will be performed on a 3 Tesla scanner at screening 2 visit to determine baseline WMH severity. We will use the standardized Canadian Dementia Imaging Protocol, which allows for semiautomatic and simultaneous quantification of regional small vessel disease, including WMH, lacunes, and perivascular spaces, as well as cerebrospinal, grey and white matter segmentation[82-87]. Sequences include high resolution 3D T1, Proton Density **(PD)**/T2 weighted sequence, fluid attenuated inversion recovery **(FLAIR**), Susceptibility Weighted Imaging (for microbleed counts). The PD/T2 images will be used for image co-registration and to generate intracranial volume mask[88]. Following tissue segmentation, regional parcellation of 26 bilateral brain regions will be generated using Semiautomatic Brain Region Extraction (**SABRE;** Sunnybrook Health Sciences Centre)[83]. FLAIR or PD/T2 images will be used to identify WMH using one of two semiautomatic protocols (Fuzzy Lesion Extractor **[FLEX][85]** or Lesion Explorer[82] with manual selection editing to obtain reliable quantification of the number, size, location, and volume of WMH lesions[82,89]. All image acquisition, processing, and scoring will be blinded to clinical information using our standardized protocol.

7.2.1 MRS Data Acquisition

MRS data will be acquired to assess GSH concentrations in vivo. The regions of interest in the brain will be chosen based on a high-quality signal and relevance to the cognitive assessments outlined in section 7.1. The MEGA-PRESS pulse sequence will be used, enabling expression of GSH levels as an absolute concentration in mM.

7.3 Blood Samples

Blood samples will be collected (schedule shown in Appendix A) to measure plasma levels of GSH antioxidant system and serum levels of lipid peroxidation as previously described [15,90]. Briefly, approximately four teaspoons of blood will be collected by antecubital venipuncture from each study participant following at least 12 hours of fasting. Blood draws will be scheduled for 0900 h $\pm$ 3 h to minimize the effect of diurnal or dietary variation on marker concentrations. Blood will be drawn into SST and EDTA-containing vacutainer tubes and centrifuged (The Drucker Company; Model 614B) at 1000 x g for 10 minutes. Plasma and serum will be isolated and frozen at -80 C. Stored samples will be batched for analysis. **GSH antioxidant system:** We will measure levels of GPx, GR and GST using standard western blotting technique with appropriate primary and secondary antibodies[15]. **S100B Assay:** We will measure levels of S100B (pg/mL) using a standard sandwich immunosorbent assay (ELISA). **LPH Assay:** Early-stage oxidative damage to lipids will be evaluated by measuring the levels of LPH (μM) using the colorimetric LPH assay kit (Cayman Chemical; Item No. 705002) according to manufacturer's instructions. **4-HNE Assay:** Late-stage lipid peroxidation will be assessed by measuring 4-HNE (fmol/μg) (Cell Biolabs, Inc.; STA-338), which will be quantified using a standard sandwich ELISA designed to detect protein adducts formed via 4-HNE Michael Addition to lysine, histidine, or cysteine residues. **8-ISO Assay:** Late-stage lipid peroxidation will be evaluated by measuring 8-ISO (pg/mL) (Cayman Chemical; Item No. 516351), which will be quantified with a standard competitive sandwich ELISA using an 8-ISO-acetylcholinesterase conjugate as a tracer and 8-ISO-specific rabbit antiserum. The sensitivity and intra- and inter- assay coefficient of variation (CV) of these assays have been validated and published previously[91]. **Oxylipins:** Oxylipin species will be assayed by ultra-high-pressure liquid chromatography tandem mass spectrometry[92,93]. **MPO assay**: MPO concentrations in blood (pg/mL) will be measured using ELISA. **Ccf-mtDNA**: Mitochondrial dysfunction will be assessed by ccf-mtDNA concentrations, which will be measured using quantitative polymerase chain reaction from circulating cell free DNA purified by commercially available spin columns. **PIGF**: We will measure levels of PlGF using a standard sandwich ELISA. Blood will also be collected at baseline and analyzed for the APOE genotype. Blood cells will be frozen separately for determination of APOE genotype. ApoE genotype will be determined by a polymerase chain reaction based assay[94] at Sunnybrook.

7.4 Statistical Analyses

All analyses will be performed using current versions of SPSS and SAS (SAS Institute, Cary, NC, USA). The intention-to-treat analyses will be based on data from all patients who undergo randomization, meet the inclusion criteria for the study, receive the trial medication at least once, and undergo at least baseline observation. Descriptive statistics will be calculated for composite z-scores for executive function, memory, and speed of processing. Categorical variables will be summarized using counts and percentages. Continuous variables will be compared between groups using two sample two sided t-tests (or Wilcoxon rank sum tests for non-normal data), whereas categorical variables will be compared between groups using chi-square analyses or Fisher's exact tests.

Primary Hypothesis: Differences in executive function composite z scores between the groups at 24 weeks will be compared between treatment groups using a two sample two sided t-test. A linear mixed model will be run to compare the rate of change in the executive function measure over time (baseline, 12- and 24-weeks). The linear mixed model is selected as it can accommodate missing data in subjects. The effects of age, sex, BMI, total years of education, VO_2 peak, physical activity levels, serum homocysteine, will be included as possible covariates. The same analyses will be repeated with the composite z-scores of the other cognitive domains as repeated variables to test for the effect of NAC treatment on the secondary outcome measures.

Secondary Hypotheses: A linear mixed model will also be used to test the hypothesis that patients with mild VCI treated with NAC will show a greater reduction in 8-ISO/LPH ratio over 24 weeks of treatment. The early to late stage lipid peroxidation marker ratio will be inputted as the repeated variable, and treatment group will be added as a fixed factor. The effects of group by time interaction will be evaluated to indicate significant effects of NAC in reducing oxidative stress markers as compared to placebo. APOE4 genotype status, BMI, VO_2 peak, and sex will be added as covariates. Similar analyses will be conducted separately with GPx, GR and GST inputted as the repeated variable. Logistic regression analyses will be performed to examine whether baseline severity of WMH is a significant predictor of cognitive response to NAC treatment at 24 weeks, adjusting for the same covariates.

Exploratory Hypothesis:
A linear mixed model will be used to assess baseline brain GSH as a predictor of change in executive function, processing speed, memory and working memory composite z scores over 24 weeks in patients with mild VCI. A GSH x time interaction will indicate a significant relationship between brain GSH and change in executive function, processing speed, memory and working memory performance over time.

Post-hoc analyses will be used to assess change in 8-ISO/LPH ratio over 24 weeks of treatment between possible and probable mild VCI subgroups using linear mixed models. Between group differences in baseline brain GSH levels will also be assessed using analyses of covariance. Where possible, the primary and secondary hypotheses will also be tested in the probable and possible mild VCI subgroups and compared.

Linear regression will be used to investigate baseline serum S100B as a predictor of baseline brain GSH levels, and if serum S100B at baseline will predict change in brain GSH over a 6-month period. Body fat percentage will be included as a potential confounder in this analysis.

Linear regression will be used to assess association between mitochondrial dysfunction as measured by ccf-mtDNA and oxidative stress markers at baseline and over time. A linear regression model will be used to investigate the association between PlGF, oxidative stress markers at baseline and termination. A linear regression model will be used to investigate peripheral MPO as a predictor of executive function or verbal memory at baseline. Age, use of anti-inflammatory medications and inflammatory and vascular comorbidities will be included as covariates. Measures of mood, cognitive function, and metabolic markers (oxylipins) will be compared between the baseline and follow-up visits, and changes in these measures will be compared between individuals relative to each other in a repeated measures linear regression analyses.

Safety Outcomes: Frequency of adverse events between treatment and placebo groups will be compared using chi-square/ Fisher exact tests. In addition, exploratory analyses using logistic regression will be performed to determine predictors of cognitive response to NAC, including demographics and compliance.

7.5 Power Calculation

Based on a published randomized placebo-controlled trial in patients with probable AD, oral NAC treatment resulted in a significant improvement in executive function in the active treatment group (p= 0.008) but not in placebo[38]. Our preliminary analysis of MOVE-IT blinded data (n=30) was used to provide an updated estimate of the standard deviation (SD=0.55) of executive function Z-scores in our population, controlling for education. Based on this, a sample size of 42 (21 in each group) will provide 80% power, at alpha of 0.05, to detect a medium effect size in a linear mixed model with a hypothesized intra-cluster correlation of 0.1. The sample size calculation was carried out using PASS Version 12 (Kaysville, Utah.). Due to the uncertainties of COVID-19 pandemic, a generous drop-out rate was used. To account for a drop-out rate of 42%, we will inflate the sample size to 60.

Protocol Deviations

Planned deviations from the protocol will not be implemented without prior agreement from the sponsor and approval from Sunnybrook Health Sciences Centre REB, unless to eliminate an immediate hazard to a participant. Protocol deviations will be documented and reported as required and assessed where necessary during statistical analysis.

8 ASSESSMENT OF SAFETY

The safety of research participants is foremost and should always be considered throughout the conduct of research.

8.1 Definitions

8.1.1 Adverse Events

An adverse event (AE) means any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment, and includes an adverse drug reaction (ADR).

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

8.1.2 Serious Adverse Events

A serious adverse event (SAE) or reaction is any untoward occurrence that at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or in significant disability/incapacity,
- Is a congenital abnormality or a birth defect.

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

8.1.3 Unexpected Adverse Event

An unexpected adverse event is any AE that is not identified in nature, severity or frequency in the current Product Monograph.

8.1.4 Unexpected Adverse Drug Reaction (ADR)

An ADR is an adverse reaction, the severity of which is not consistent with the applicable Product Monograph. All noxious and unintended responses to a medicinal product related to any dose should be considered an ADR.

The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

The expression “causal relationship” is meant to convey that in general there are facts, evidence or arguments to suggest a reasonable causal relationship. All serious and unexpected ADRs will have expedited reporting to regulatory agencies following ICH-GCP and local regulatory requirements.

8.2 Assessment of an Adverse Event

8.2.1 Relationship (Causality/Relatedness)

The causality assessment is the determination, according to the investigator's clinical judgment, of the existence of a reasonable possibility that the study drug (IP) caused or contributed to an adverse event.

If the qualified investigator or delegated sub-investigator is unsure about whether or not the study drug caused or is related to the event, then the event will be handled as "related" to the study drug for reporting purposes of the trial. If the causality assessment is "unknown but not related" to the study drug, this will be clearly documented in the source documents.

8.2.2 Expectedness

Events will be classified as unforeseen or unexpected if the nature, severity or frequency is not consistent with the risk information set out in the Product Monograph or label.

8.2.3 Seriousness

Events will be classified as serious if associated with effects threatening the life or physiological functions of a participant. Refer to the definition for "Serious Adverse Events" in section 8.1.2.

8.2.4 Severity

The term "severe" is often used to describe the intensity (severity) of a specific event (e.g. mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on participant/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. The terms "serious" and "severe" are not synonymous. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.3 Adverse Event Recording

Investigations into potential adverse events will be conducted throughout the study (in person during the baseline visit, and at 4, 8, 12, 16, 20 and 24 weeks; and over the phone as shown on Appendix A) using a symptom checklist and will be recorded promptly in the source document. This checklist will include all potential side effects listed on the current product monograph for NAC. All emerging AEs will be noted and followed-up until resolution in a timely manner.

8.3.1 Investigator reporting: Notifying the REB

Serious adverse events and unanticipated events will be recorded and reported to the REB in accordance with local reporting requirements and timelines.

8.3.2 Investigator reporting: Notifying the Sponsor

The investigator will report serious adverse events and serious and unexpected adverse drug reactions (SUADRs) to the sponsor in accordance with applicable regulations and reporting requirements and timelines.

8.3.3 Sponsor Reporting of SUADRs: Notifying Health Canada

The regulatory sponsor will report SUADRs to regulatory authorities in accordance with local expedited reporting requirements and timelines. In addition, the Sponsor will complete the ADR Expedited Reporting Summary Form and submit this form in conjunction with the completed CIOMS Form to the appropriate Health Canada directorate..

8.3.4 Sponsor Reporting of SUADRs: Notifying Sites

The regulatory sponsor is responsible for distributing blinded expedited reports of SUADRs to each investigator for submission to local Ethics Committees within 15 days of sponsor awareness.

8.4 Type and Duration of Follow-up for Adverse Events

AEs occurring as of the first administered dose of the investigational product and to a follow up period of at least 32 hours (half life of NAC is 6.25hrs)[31] after the last administered dose, will be collected. AEs recorded during this period will be followed through to resolution, or until the event is assessed as chronic or stable.

8.5 Reporting and Entry Timelines

The principal investigator will report SAEs to the sponsor within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded and reported to the sponsor within 24 hours of site awareness.
- Serious adverse events other than death and immediately life-threatening events, regardless of relationship, will be reported to the sponsor within 72 hours of site awareness.

Adverse event information will be entered into the CRF in a timely manner and **no later than 15 days** from the time the investigator becomes aware of the event.

Serious adverse event information will be entered into the CRF in a timely manner/**within 72 hours** from the time the investigator becomes aware of the event.

8.6 Rescue Medication

In case of overdose, the managements should be focused on the treatment of symptoms. These symptoms should be managed according to current standards of care with general supportive measures. Also discontinuation of study drug should be considered.

9 SITE MONITORING, AUDITING AND INSPECTING

9.1 Site Monitoring Plan

The extent and nature of monitoring is outlined in the Study Monitoring Plan. Monitoring activities will be performed both in person and remotely. Reports of findings identified during monitoring activities will be provided to sites detailing any required actions. Documentation of monitoring activities and findings will be provided to the site study team and the study QI. The institution and/or local REB reserves the right to conduct independent audits as necessary.

9.2 Auditing and Inspecting

The investigator will provide direct access to source data/documents for the purposes of study-related monitoring, audits, and inspections by the REB, the sponsor, and applicable regulatory bodies. The investigator will permit the review of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.) and will ensure access to applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

10 DATA HANDLING AND RECORD KEEPING

10.1 Confidentiality

Information about study participants will be kept confidential and managed according to the requirements of the Personal Health Information Protection Act of 2004 (PHIPA) and the Research Ethics Board. PHIPA outlines the rules for the collection, use and disclosure of personal health information.

10.2 Source Documents

The investigator(s) and research team members listed on the Task Delegation Log will have access to participant medical records and will collect only the information needed for the study. Sponsor delegated monitors, representatives of institutional committees and regulatory authority representatives will also have access to examine records for the purposes of quality assurance reviews, audits and evaluation of study safety and progress.

Data will be collected on printed source documents/case report forms (**CRFs**). Source documents/CRFs include physical, cognitive, and clinician assessments.

10.3 Data Management Responsibilities

As part of the safety plan for this study, the investigator will review individual study participant records to ensure that appropriate mechanisms to protect the safety of study participants are being followed, that protocol requirements are being adhered to, and that data is accurate, complete, and secure. Participant records include, but are not limited to: consent forms, case report forms, data forms, laboratory records, inclusion/exclusion forms, and medical charts. All study data will be collected by a member of the study research team and recorded in accordance with applicable procedures.

10.4 Data Capture

10.4.1 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. Paper case report forms (pCRFs) will be used to collect data for this study. CRFs will be completed by data capture personnel and signed off by the investigator in a timely manner. Data from pCRFs will be electronically entered into Research Electronic Data Capture (REDCap). Good documentation practices will be implemented according to standard operating procedures. All data reported on the CRF will be verifiable by source document.

10.5 Records Retention

Study essential documents will be maintained in a secure and confidential manner for a period of 25 years. For the purposes of this study, the start date of the retention period will be the date of the final report of the trial. All study records will be destroyed according to local and national policy and requirements. The sponsor will inform the investigator/institution when these documents may be destroyed.

10.6 Clinical Trial Registration

In accordance with Health Canada's Notice "Registration and Disclosure of Clinical Trial Information, November 30, 2007", the study will be registered on Clinicaltrials.gov (*www.clinicaltrials.gov*).

11 ETHICS CONSIDERATIONS

11.1 Ethical Standard

This study will be conducted in accordance with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Participants of Research, and codified in the Tri-Council Policy Statement and/or the ICH E6.

11.2 Research Ethics Board (REB)

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the REB for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is enrolled. Any amendment to the protocol will be approved by the REB before the changes are implemented in the study, unless to eliminate an immediate hazard.

11.3 Consent

A consent form describing in detail the study procedures and risks will be reviewed with and given to each participant. Consent forms will be REB-approved, and the participant will be required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent form prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records.

12 PANDEMIC CONTINGENCY PLAN

12.1 Alternative Methods for Consenting

In order to reduce risk to study participants and staff in pandemic situation (e.g COVID-19), alternative consenting methods such as videoconference or telephone consents is available to participants.

12.1.1 Consent obtained remotely by phone or video conference

The research team will arrange a three-way call or video conference with the participant, an impartial witness, and if desired and feasible, additional participants requested by the participant (e.g., next of kin). Research staff will mail or email the participant a form ahead of time, explain the consent forms and answer any question during the call or video conference. The consent forms will be signed by the participant and a witness, and sent back to the research team via mail or email. Research team will have PI review and sign the consent form, provide the participant with a copy for their records. The process will be documented.

12.2 Remote Assessments

A schedule to reduce in-person contact and risk is provided in Appendix B. Participants will complete Baseline visit, Week 4, Week 8, Week 16 and Week 20 visits remotely by phone or video conference.

12.3 Investigational Product Administration and Monitoring

Participants will receive and return IP via tracked shipment. The research team will give phone calls regularly to follow up with the participants and monitor any possible adverse event. Compliance will be monitored using capsule counts.

12.4 Blood Samples

Blood samples will be collected at Screening 1 and Screening 2 visits (shown in Appendix B) instead of Screening 2 and Baseline visit to reduce the time that the participants have to come on-site.

13 BIBLIOGRAPHY

1. Rockwood K, Wentzel C, Hachinski V, Hogan DB, MacKnight C, McDowell I. Prevalence and outcomes of vascular cognitive impairment. Vascular Cognitive Impairment Investigators of the Canadian Study of Health and Aging. *Neurology*. Jan 25 2000;54(2):447-51.
2. Skrobot OA, Black SE, Chen C, et al. Progress toward standardized diagnosis of vascular cognitive impairment: Guidelines from the Vascular Impairment of Cognition Classification Consensus Study. *Alzheimer's & Dementia*. 2018/03/01/ 2018;14(3):280-292. doi:*https://doi.org/10.1016/j.jalz.2017.09.007*
3. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke*. Sep 2011;42(9):2672-713. doi:10.1161/STR.0b013e3182299496
4. Fitzpatrick-Lewis D, Warren R, Ali MU, Sherifali D, Raina P. Treatment for mild cognitive impairment: a systematic review and meta-analysis. *CMAJ Open*. Oct-Dec 2015;3(4):E419-27. doi:10.9778/cmajo.20150057
5. Peters R, Beckett N, Forette F, et al. Incident dementia and blood pressure lowering in the Hypertension in the Very Elderly Trial cognitive function assessment (HYVET-COG): a double-blind, placebo controlled trial. *Lancet Neurol*. Aug 2008;7(8):683-9. doi:10.1016/S1474-4422(08)70143-1
6. Raschetti R, Albanese E, Vanacore N, Maggini M. Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials. *PLoS Med*. Nov 27 2007;4(11):e338. doi:10.1371/journal.pmed.0040338
7. Zheng G, Xia R, Zhou W, Tao J, Chen L. Aerobic exercise ameliorates cognitive function in older adults with mild cognitive impairment: a systematic review and meta-analysis of randomised controlled trials. *Br J Sports Med*. Apr 19 2016;doi:10.1136/bjsports-2015-095699
8. Verdelho A, Madureira S, Ferro JM, et al. Physical activity prevents progression for cognitive impairment and vascular dementia: results from the LADIS (Leukoaraiosis and Disability) study. *Stroke*. Dec 2012;43(12):3331-5. doi:10.1161/STROKEAHA.112.661793
9. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci*. Mar 2003;14(2):125-30.
10. Gates N, Fiatarone Singh MA, Sachdev PS, Valenzuela M. The effect of exercise training on cognitive function in older adults with mild cognitive impairment: a meta-analysis of randomized controlled trials. *Am J Geriatr Psychiatry*. Nov 2013;21(11):1086-97. doi:10.1016/j.jagp.2013.02.018
11. Young J, Angevaren M, Rusted J, Tabet N. Aerobic exercise to improve cognitive function in older people without known cognitive impairment. *Cochrane Database Syst Rev*. 2015;(4):CD005381. doi:10.1002/14651858.CD005381.pub4
12. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. *Nat Rev Drug Discov*. Mar 2004;3(3):205-14. doi:10.1038/nrd1330
13. Bains JS, Shaw CA. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain Res Brain Res Rev*. Dec 1997;25(3):335-58.
14. Pratico D, Sung S. Lipid peroxidation and oxidative imbalance: early functional events in Alzheimer's disease. *J Alzheimers Dis*. Apr 2004;6(2):171-5.
15. Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *Int J Neuropsychopharmacol*. Feb 2011;14(1):123-30. doi:10.1017/S1461145710000805
16. Pocernich CB, Lange ML, Sultana R, Butterfield DA. Nutritional approaches to modulate oxidative stress in Alzheimer's disease. *Curr Alzheimer Res*. Aug 2011;8(5):452-69.
17. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. *JAMA*. Jun 26 2002;287(24):3230-7.

18. Zandi PP, Anthony JC, Khachaturian AS, et al. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. *Arch Neurol*. Jan 2004;61(1):82-8. doi:10.1001/archneur.61.1.82
19. Devore EE, Grodstein F, van Rooij FJ, et al. Dietary antioxidants and long-term risk of dementia. *Arch Neurol*. Jul 2010;67(7):819-25. doi:10.1001/archneurol.2010.144
20. Mecocci P, Polidori MC. Antioxidant clinical trials in mild cognitive impairment and Alzheimer's disease. *Biochim Biophys Acta*. May 2012;1822(5):631-8. doi:10.1016/j.bbadis.2011.10.006
21. Pocernich CB, Butterfield DA. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. *Biochim Biophys Acta*. May 2012;1822(5):625-30. doi:10.1016/j.bbadis.2011.10.003
22. Lopes da Silva S, Vellas B, Elemans S, et al. Plasma nutrient status of patients with Alzheimer's disease: Systematic review and meta-analysis. *Alzheimers Dement*. Jul 2014;10(4):485-502. doi:10.1016/j.jalz.2013.05.1771
23. Pratico D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. *Arch Neurol*. Jun 2002;59(6):972-6.
24. Markesbery WR, Carney JM. Oxidative alterations in Alzheimer's disease. *Brain Pathol*. Jan 1999;9(1):133-46.
25. Subbarao KV, Richardson JS, Ang LC. Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. *J Neurochem*. Jul 1990;55(1):342-5.
26. Lovell MA, Ehmann WD, Butler SM, Markesbery WR. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology*. Aug 1995;45(8):1594-601.
27. Marcus DL, Thomas C, Rodriguez C, et al. Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Exp Neurol*. Mar 1998;150(1):40-4. doi:10.1006/exnr.1997.6750
28. Jeandel C, Nicolas MB, Dubois F, Nabet-Belleville F, Penin F, Cuny G. Lipid peroxidation and free radical scavengers in Alzheimer's disease. *Gerontology*. 1989;35(5-6):275-82.
29. de Sousa CV, Sales MM, Rosa TS, Lewis JE, de Andrade RV, Simoes HG. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. *Sports Med*. Jun 3 2016;doi:10.1007/s40279-016-0566-1
30. Borgstrom L, Kagedal B. Dose dependent pharmacokinetics of N-acetylcysteine after oral dosing to man. *Biopharm Drug Dispos*. Mar 1990;11(2):131-6.
31. Holdiness MR. Clinical pharmacokinetics of N-acetylcysteine. *Clin Pharmacokinet*. Feb 1991;20(2):123-34. doi:10.2165/00003088-199120020-00004
32. Olsson B, Johansson M, Gabrielsson J, Bolme P. Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. *Eur J Clin Pharmacol*. 1988;34(1):77-82.
33. Farr SA, Poon HF, Dogrukol-Ak D, et al. The antioxidants alpha-lipoic acid and N-acetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice. *J Neurochem*. Mar 2003;84(5):1173-83.
34. Lavoie S, Murray MM, Deppen P, et al. Glutathione precursor, N-acetyl-cysteine, improves mismatch negativity in schizophrenia patients. *Neuropsychopharmacology*. Aug 2008;33(9):2187-99. doi:10.1038/sj.npp.1301624
35. Slattery KM, Dascombe B, Wallace LK, Bentley DJ, Coutts AJ. Effect of N-acetylcysteine on cycling performance after intensified training. *Med Sci Sports Exerc*. Jun 2014;46(6):1114-23. doi:10.1249/MSS.0000000000000222
36. Erickson MA, Hansen K, Banks WA. Inflammation-induced dysfunction of the low-density lipoprotein receptor-related protein-1 at the blood-brain barrier: protection by the antioxidant N-acetylcysteine. *Brain Behav Immun*. Oct 2012;26(7):1085-94. doi:10.1016/j.bbi.2012.07.003

37. Fu AL, Dong ZH, Sun MJ. Protective effect of N-acetyl-L-cysteine on amyloid beta-peptide-induced learning and memory deficits in mice. *Brain Res*. Sep 13 2006;1109(1):201-6. doi:10.1016/j.brainres.2006.06.042
38. Adair JC, Knoefel JE, Morgan N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. *Neurology*. Oct 23 2001;57(8):1515-7.
39. Remington R, Bechtel C, Larsen D, et al. A Phase II Randomized Clinical Trial of a Nutritional Formulation for Cognition and Mood in Alzheimer's Disease. *J Alzheimers Dis*. 2015;45(2):395-405. doi:10.3233/JAD-142499
40. Remington R, Lortie JJ, Hoffmann H, Page R, Morrell C, Shea TB. A Nutritional Formulation for Cognitive Performance in Mild Cognitive Impairment: A Placebo-Controlled Trial with an Open-Label Extension. *J Alzheimers Dis*. 2015;48(3):591-5. doi:10.3233/JAD-150057
41. Remington R, Bechtel C, Larsen D, et al. Maintenance of Cognitive Performance and Mood for Individuals with Alzheimer's Disease Following Consumption of a Nutraceutical Formulation: A One-Year, Open-Label Study. *J Alzheimers Dis*. 2016;51(4):991-5. doi:10.3233/JAD-151098
42. Vasquez BP, Zakzanis KK. The neuropsychological profile of vascular cognitive impairment not demented: a meta-analysis. *J Neuropsychol*. Mar 2015;9(1):109-36. doi:10.1111/jnp.12039
43. Lovell MA, Markesbery WR. Oxidative damage in mild cognitive impairment and early Alzheimer's disease. *J Neurosci Res*. Nov 1 2007;85(14):3036-40. doi:10.1002/jnr.21346
44. Strohle A, Schmidt DK, Schultz F, et al. Drug and Exercise Treatment of Alzheimer Disease and Mild Cognitive Impairment: A Systematic Review and Meta-Analysis of Effects on Cognition in Randomized Controlled Trials. *Am J Geriatr Psychiatry*. Dec 2015;23(12):1234-49. doi:10.1016/j.jagp.2015.07.007
45. Moreira PI, Harris PL, Zhu X, et al. Lipoic acid and N-acetyl cysteine decrease mitochondrial-related oxidative stress in Alzheimer disease patient fibroblasts. *J Alzheimers Dis*. Sep 2007;12(2):195-206.
46. Xu X, Chan QL, Hilal S, et al. The Diagnostic Utility of the NINDS-CSN Neuropsychological Battery in Memory Clinics. *Dement Geriatr Cogn Dis Extra*. May-Aug 2016;6(2):276-82. doi:10.1159/000445050
47. Bloch MH, Panza KE, Grant JE, Pittenger C, Leckman JF. N-Acetylcysteine in the treatment of pediatric trichotillomania: a randomized, double-blind, placebo-controlled add-on trial. *J Am Acad Child Adolesc Psychiatry*. Mar 2013;52(3):231-40. doi:10.1016/j.jaac.2012.12.020
48. Gray KM, Carpenter MJ, Baker NL, et al. A double-blind randomized controlled trial of N-acetylcysteine in cannabis-dependent adolescents. *Am J Psychiatry*. Aug 2012;169(8):805-12. doi:10.1176/appi.ajp.2012.12010055
49. Berk M, Copolov D, Dean O, et al. N-acetyl cysteine as a glutathione precursor for schizophrenia--a double-blind, randomized, placebo-controlled trial. *Biol Psychiatry*. Sep 01 2008;64(5):361-8. doi:10.1016/j.biopsych.2008.03.004
50. Dean OM, Bush AI, Copolov DL, et al. Effects of N-acetyl cysteine on cognitive function in bipolar disorder. *Psychiatry Clin Neurosci*. Oct 2012;66(6):514-7. doi:10.1111/j.1440-1819.2012.02392.x
51. Holmay MJ, Terpstra M, Coles LD, et al. N-Acetylcysteine boosts brain and blood glutathione in Gaucher and Parkinson diseases. *Clin Neuropharmacol*. Jul-Aug 2013;36(4):103-6. doi:10.1097/WNF.0b013e31829ae713
52. Ho E, Karimi Galougahi K, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox Biol*. 2013;1:483-91. doi:10.1016/j.redox.2013.07.006
53. Sudo FK, Alves CE, Alves GS, et al. White matter hyperintensities, executive function and global cognitive performance in vascular mild cognitive impairment. *Arq Neuropsiquiatr*. Jul 2013;71(7):431-6. doi:10.1590/0004-282X20130057

54. Tullberg M, Fletcher E, DeCarli C, et al. White matter lesions impair frontal lobe function regardless of their location. *Neurology*. Jul 27 2004;63(2):246-53.
55. Schmidt R, Petrovic K, Ropele S, Enzinger C, Fazekas F. Progression of leukoaraiosis and cognition. *Stroke*. Sep 2007;38(9):2619-25. doi:10.1161/STROKEAHA.107.489112
56. Demedts M, Behr J, Buhl R, et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med*. Nov 24 2005;353(21):2229-42. doi:10.1056/NEJMoa042976
57. Katz M, Won SJ, Park Y, et al. Cerebrospinal fluid concentrations of N-acetylcysteine after oral administration in Parkinson's disease. *Parkinsonism Relat Disord*. May 2015;21(5):500-3. doi:10.1016/j.parkreldis.2015.02.020
58. Chin J, Seo SW, Kim SH, et al. Neurobehavioral dysfunction in patients with subcortical vascular mild cognitive impairment and subcortical vascular dementia. *Clin Neuropsychol*. 2012;26(2):224-38. doi:10.1080/13854046.2012.658865
59. Ismail Z, Aguera-Ortiz L, Brodaty H, et al. The Mild Behavioral Impairment Checklist (MBI-C): A Rating Scale for Neuropsychiatric Symptoms in Pre-Dementia Populations. *J Alzheimers Dis*. 2017;56(3):929-938. doi:10.3233/JAD-160979
60. Hsieh CJ, Chu H, Cheng JJ, Shen WW, Lin CC. Validation of apathy evaluation scale and assessment of severity of apathy in Alzheimer's disease. *Psychiatry Clin Neurosci*. Apr 2012;66(3):227-34. doi:10.1111/j.1440-1819.2011.02315.x
61. Yu D, Hennebelle M, Sahlas DJ, et al. Soluble Epoxide Hydrolase-Derived Linoleic Acid Oxylipins in Serum Are Associated with Periventricular White Matter Hyperintensities and Vascular Cognitive Impairment. *Transl Stroke Res*. Oct 2019;10(5):522-533. doi:10.1007/s12975-018-0672-5
62. Nayeem MA. Role of oxylipins in cardiovascular diseases. *Acta Pharmacol Sin*. Jul 2018;39(7):1142-1154. doi:10.1038/aps.2018.24
63. Hammadah M, Georgiopoulou VV, Kalogeropoulos AP, et al. Elevated Soluble Fms-Like Tyrosine Kinase-1 and Placental-Like Growth Factor Levels Are Associated With Development and Mortality Risk in Heart Failure. *Circ Heart Fail*. Jan 2016;9(1):e002115. doi:10.1161/circheartfailure.115.002115
64. Ribatti D. The discovery of the placental growth factor and its role in angiogenesis: a historical review. *Angiogenesis*. 2008;11(3):215-21. doi:10.1007/s10456-008-9114-4
65. Landers-Ramos RQ, Jenkins NT, Spangenburg EE, Hagberg JM, Prior SJ. Circulating angiogenic and inflammatory cytokine responses to acute aerobic exercise in trained and sedentary young men. *Eur J Appl Physiol*. 2014;114(7):1377-84. doi:10.1007/s00421-014-2861-6
66. Schroeter ML, Sacher J, Steiner J, Schoenknecht P, Mueller K. Serum S100B represents a new biomarker for mood disorders. *Curr Drug Targets*. 2013;14(11):1237-1248. doi:10.2174/13894501131491990014
67. Petzold A, Jenkins R, Watt HC, et al. Cerebrospinal fluid S100B correlates with brain atrophy in Alzheimer's disease. *Neurosci Lett*. Jan 23 2003;336(3):167-70. doi:S0304394002012570 [pii]
68. Chaves ML, Camozzato AL, Ferreira ED, et al. Serum levels of S100B and NSE proteins in Alzheimer's disease patients. *J Neuroinflammation*. 2010;7:6-6. doi:10.1186/1742-2094-7-6
69. Cristóvão JS, Gomes CM. S100 Proteins in Alzheimer’s Disease. Review. *Frontiers in Neuroscience*. 2019-May-16 2019;13(463)doi:10.3389/fnins.2019.00463
70. Dong Y, Lagarde J, Xicota L, et al. Neutrophil hyperactivation correlates with Alzheimer's disease progression. *Ann Neurol*. Feb 2018;83(2):387-405. doi:10.1002/ana.25159
71. Bawa KK, Krance SH, Herrmann N, et al. A peripheral neutrophil-related inflammatory factor predicts a decline in executive function in mild Alzheimer's disease. *J Neuroinflammation*. Mar 14 2020;17(1):84. doi:10.1186/s12974-020-01750-3
72. Teng N, Maghzal GJ, Talib J, Rashid I, Lau AK, Stocker R. The roles of myeloperoxidase in coronary artery disease and its potential implication in plaque rupture. *Redox Rep*. Mar 2017;22(2):51-73. doi:10.1080/13510002.2016.1256119

73. Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. *Jama*. Nov 7 2001;286(17):2136-42. doi:10.1001/jama.286.17.2136
74. Meuwese MC, Stroes ES, Hazen SL, et al. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. *J Am Coll Cardiol*. Jul 10 2007;50(2):159-65. doi:10.1016/j.jacc.2007.03.033
75. Wright CB, Moon Y, Paik MC, et al. Inflammatory biomarkers of vascular risk as correlates of leukoariosis. *Stroke*. Nov 2009;40(11):3466-71. doi:10.1161/strokeaha.109.559567
76. Tzikas S, Schlak D, Sopova K, et al. Increased myeloperoxidase plasma levels in patients with Alzheimer's disease. *J Alzheimers Dis*. 2014;39(3):557-64. doi:10.3233/jad-131469
77. Sachdev P, Kalaria R, O'Brien J, et al. Diagnostic criteria for vascular cognitive disorders: a VASCOG statement. *Alzheimer Dis Assoc Disord*. Jul-Sep 2014;28(3):206-18. doi:10.1097/WAD.0000000000000034
78. Wahlund LO, Barkhof F, Fazekas F, et al. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke*. Jun 2001;32(6):1318-22.
79. Dufouil C, Tzourio C, Brayne C, Berr C, Amouyel P, Alperovitch A. Influence of apolipoprotein E genotype on the risk of cognitive deterioration in moderate drinkers and smokers. *Epidemiology*. May 2000;11(3):280-4.
80. Hachinski V, Iadecola C, Petersen RC, et al. National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards. *Stroke*. Sep 2006;37(9):2220-41. doi:10.1161/01.STR.0000237236.88823.47
81. Xu Q, Cao WW, Mi JH, Yu L, Lin Y, Li YS. Brief screening for mild cognitive impairment in subcortical ischemic vascular disease: a comparison study of the Montreal Cognitive Assessment with the Mini-Mental State Examination. *Eur Neurol*. 2014;71(3-4):106-14. doi:10.1159/000353988
82. Ramirez J, Gibson E, Quddus A, et al. Lesion Explorer: a comprehensive segmentation and parcellation package to obtain regional volumetrics for subcortical hyperintensities and intracranial tissue. *Neuroimage*. Jan 15 2011;54(2):963-73. doi:10.1016/j.neuroimage.2010.09.013
83. Dade LA, Gao FQ, Kovacevic N, et al. Semiautomatic brain region extraction: a method of parcellating brain regions from structural magnetic resonance images. *Neuroimage*. Aug 2004;22(4):1492-502. doi:10.1016/j.neuroimage.2004.03.023
84. Kovacevic N, Lobaugh NJ, Bronskill MJ, Levine B, Feinstein A, Black SE. A robust method for extraction and automatic segmentation of brain images. *Neuroimage*. Nov 2002;17(3):1087-100.
85. Gibson E, Gao F, Black SE, Lobaugh NJ. Automatic segmentation of white matter hyperintensities in the elderly using FLAIR images at 3T. *J Magn Reson Imaging*. Jun 2010;31(6):1311-22. doi:10.1002/jmri.22004
86. Ramirez J, McNeely AA, Scott CJ, Stuss DT, Black SE. Subcortical hyperintensity volumetrics in Alzheimer's disease and normal elderly in the Sunnybrook Dementia Study: correlations with atrophy, executive function, mental processing speed, and verbal memory. *Alzheimers Res Ther*. 2014;6(4):49. doi:10.1186/alzrt279
87. Levy-Cooperman N, Ramirez J, Lobaugh NJ, Black SE. Misclassified tissue volumes in Alzheimer disease patients with white matter hyperintensities: importance of lesion segmentation procedures for volumetric analysis. *Stroke*. Apr 2008;39(4):1134-41. doi:10.1161/STROKEAHA.107.498196
88. Woods RP, Grafton ST, Holmes CJ, Cherry SR, Mazziotta JC. Automated image registration: I. General methods and intrasubject, intramodality validation. *J Comput Assist Tomogr*. Jan-Feb 1998;22(1):139-52.
89. Ramirez J, Scott CJ, Black SE. A short-term scan-rescan reliability test measuring brain tissue and subcortical hyperintensity volumetrics obtained using the lesion explorer structural MRI processing pipeline. *Brain Topogr*. Jan 2013;26(1):35-8. doi:10.1007/s10548-012-0228-z

90. Andreazza AC, Kapczinski F, Kauer-Sant'Anna M, et al. 3-Nitrotyrosine and glutathione antioxidant system in patients in the early and late stages of bipolar disorder. *J Psychiatry Neurosci*. Jul 2009;34(4):263-71.
91. Scola G, McNamara RK, Croarkin PE, et al. Lipid peroxidation biomarkers in adolescents with or at high-risk for bipolar disorder. *J Affect Disord*. Mar 1 2016;192:176-83. doi:10.1016/j.jad.2015.12.020
92. Hennebelle M, Otoki Y, Yang J, et al. Altered soluble epoxide hydrolase-derived oxylipins in patients with seasonal major depression: An exploratory study. *Psychiatry Res*. Jun 2017;252:94-101. doi:10.1016/j.psychres.2017.02.056
93. Yang J, Schmelzer K, Georgi K, Hammock BD. Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. *Anal Chem*. Oct 1 2009;81(19):8085-93. doi:10.1021/ac901282n
94. Zivelin A, Rosenberg N, Peretz H, Amit Y, Kornbrot N, Seligsohn U. Improved method for genotyping apolipoprotein E polymorphisms by a PCR-based assay simultaneously utilizing two distinct restriction enzymes. *Clin Chem*. Sep 1997;43(9):1657-9.
95. Inzitari D, Pracucci G, Poggesi A, et al. Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ*. 2009;339:b2477. doi:10.1136/bmj.b2477

14 SUPPLEMENTAL MATERIALS

Appendix A: Study Assessment Schedule

Table 1. Schedule of Assessment												
Visit/phone calls (Weeks)	**-5**	**-1**	**0***	**1***	**2***	**3***	**4***	**8***	**12**	**16***	**20***	**24**
		± 14 days	± 14 days	± 2 days	± 2 days	± 2 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days
Informed Consent	X											
Inclusion/Exclusion Criteria	X	X	X									
Demographics												
Age, gender, education, language	X											
Concomitant medications	X		X				X	X	X	X	X	X
Vascular risk factors	X											
Anthropomorphic parameters			X[1]						X[1]			X[1]
Medical, Surgical, and Psychiatric History	X											
Cognitive Outcomes												
NINDCS-60 minute cognitive assessment.	X								X			X
MoCA, AES, MBI-C	X								X			X
Dietary Intake												
Food Diary			X				X	X	X	X	X	X
Fasting Blood Draw												
GSH markers: GPx, GR, GST			X						X			X
Ccf-mtDNA & OS markers: LPH, 4-HNE, 8-ISO, MPO, oxylipins			X						X			X
APOE4 genotype			X									
S100B, PlGF			X						X			X
Exploratory Neuroimaging Outcome												
MRI and MRS		X										
Exercise Intervention Outcome												
VO_2 peak			X[1]						X[1]			X[1]
IP Administration												
Drug Dispensing			X				X	X	X	X	X	
Compliance Measures												
Capsule Count							X	X	X	X	X	X
Safety Outcomes												
Heart rate, blood pressure			X				X	X	X	X	X	X
Plasma electrolyte panel and homocysteine level		X										X
Phone call				X	X	X						X[2]
Adverse events review			X	X	X	X	X	X	X	X	X	X

1. Collected from TRI on the visit date, as available. 2. At least 32 hours after the final dose.

Appendix B: Study Assessment Schedule

Table 1. Schedule of Assessment												
Visit/phone calls (Weeks)	**-5**	**-1**	**0***	**1***	**2***	**3***	**4***	**8***	**12**	**16***	**20***	**24**
		± 14 days	± 14 days	± 2 days	± 2 days	± 2 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days
Informed Consent	X											
Inclusion/Exclusion Criteria	X	X	X									
Demographics												
Age, gender, education, language	X											
Concomitant medications	X		X				X	X	X	X	X	X
Vascular risk factors	X											
Anthropomorphic parameters			X[1]						X[1]			X[1]
Medical, Surgical, and Psychiatric History	X											
Cognitive Outcomes												
NINDCS-60 minute cognitive assessment.	X								X			X
MoCA, AES, MBI-C	X								X			X
Dietary Intake												
Food Diary			X				X	X	X	X	X	X
Fasting Blood Draw												
GSH markers: GPx, GR, GST		X							X			X
Ccf-mtDNA & OS markers: LPH, 4-HNE, 8-ISO, MPO, oxylipins		X							X			X
APOE4 genotype		X										
S100B, PlGF		X							X			X
Exploratory Neuroimaging Outcome												
MRI and MRS		X										
Exercise Intervention Outcome												
VO_2 peak			X[1]						X[1]			X[1]
IP Administration												
Drug Dispensing			X[3]				X[3]	X[3]	X	X[3]	X[3]	
Compliance Measures												
Capsule Count							X	X	X	X	X	X
Safety Outcomes												
Heart rate, blood pressure			X				X	X	X	X	X	X
Plasma electrolyte panel and homocysteine level	X											X
Phone call				X	X	X						X[2]
Adverse events review			X	X	X	X	X	X	X	X	X	X

*Visit will be conducted remotely through videoconferencing or telephone. 1. Collected from TRI on the visit date, as available. 2. At least 32 hours after the final dose. 3. Pill bottle will be shipped and returned via tracked shipment.