Pilot Study of Pre-Ischemic Conditioning for Intracranial Atherosclerosis

Study Protocol

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1. STUDY RATIONALE

Intracranial atherosclerotic arterial stenosis (ICAS) is an important cause of stroke in the US, and is especially prevalent in blacks, Asians, and Hispanics.[1-6] Over the past 20 years, Dr. Chimowitz, one of the Co-PIs of this pilot study has led consecutive NIH funded multicenter clinical trials to test therapies for preventing stroke in subjects with ICAS: The WASID trial showed that aspirin was as effective as warfarin for preventing stroke, and the SAMMPRIS trial showed that aggressive medical management (AMM) consisting of dual antiplatelet therapy and intensive risk factor management was more effective than percutaneous transluminal stenting for preventing stroke in recently symptomatic subjects with ICAS.[7-9] However, among high-risk subjects (those with severe [70-99%] stenosis who presented with a stroke [rather than a TIA]), the 1-yr rate of recurrent stroke was 20.7% despite aggressive medical management.[10] Therefore, more effective therapies to prevent stroke in these high-risk patients remains an urgent need.

One promising novel treatment is remote limb ischemic conditioning (RLIC), which involves producing repetitive, transient, non-injurious ischemia of a limb by inflating a blood pressure cuff proximally with the intention of protecting a distal organ (in this case, the brain) from subsequent ischemia.[11] One of the co-investigators in our group (Dr. David Hess at the Medical College of Georgia (MCG)) has conducted pre-clinical studies using a bilateral carotid artery stenosis mouse model that showed RLIC increases cerebral blood flow (CBF), most likely by releasing nitrite into the circulation, and acts as a powerful neuroprotectant and vasculoprotectant.[12-15] Additionally, two pilot randomized clinical trials performed in China showed that symptomatic subjects with ICAS treated with bilateral upper extremity RLIC daily for 180 or 300 days and medical management had a 70-75% lower rate of stroke compared with subjects treated with medical management alone.[16,17] Although these preclinical studies and the two small Chinese pilot studies might suggest that the next step is to perform a multicenter randomized Phase II trial to determine the potential efficacy of RLIC in high-risk subjects with ICAS in the US, the lack of reliable data on how RLIC works in humans mandates an initial earlier phase trial to study potential mechanisms of action of RLIC in subjects with ICAS.

2. PRIMARY AIMS AND LONG-TERM GOAL

As a first step to collect preliminary data on the potential mechanisms of action of RLIC in humans, we will perform a small multicenter randomized pilot clinical trial of 10 subjects with ICAS at 4 participating sites (MUSC, MCG, UCLA and the University of Southern California (USC)) to accomplish the following **primary aims**:

(1) Estimate the variability in CBF (measured by arterial spin labeling and perfusion MRI) and levels of putative biomarkers of the conditioning response (vasodilatory, inflammatory, fibrinolytic, microRNA) in the two treatment groups at enrollment and after 30 days of treatment.

(2) Compare the changes in CBF and putative blood biomarkers of the conditioning response from enrollment to 30 days between the two treatment groups.

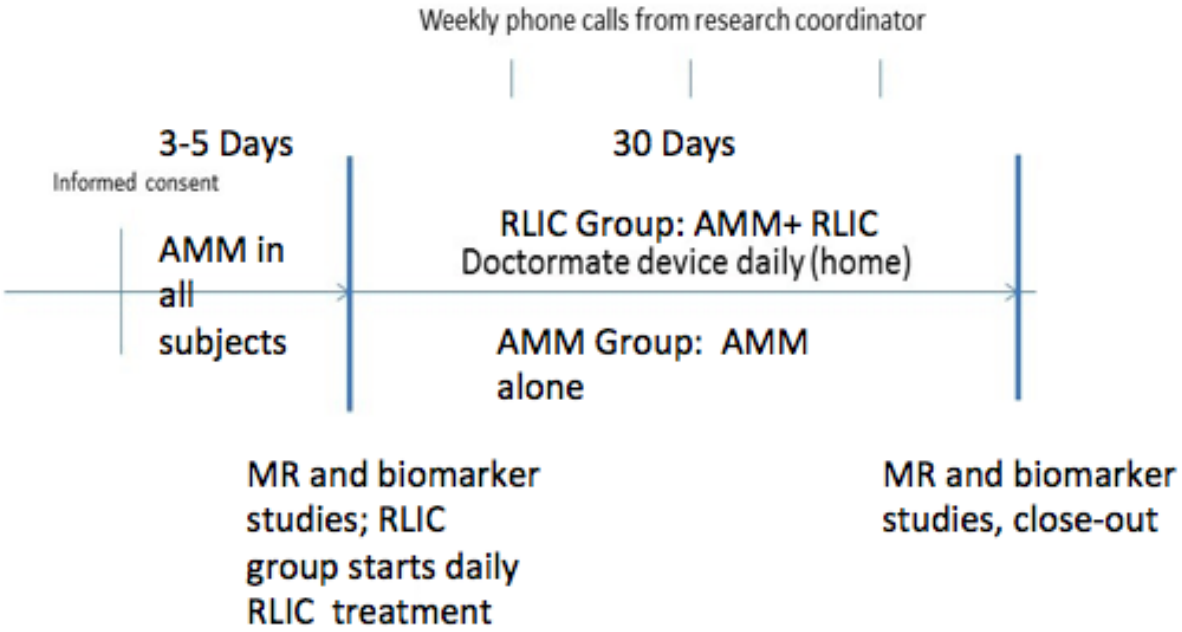
(3) Obtain preliminary feasibility, tolerability and adherence data on the use of a RLIC device for secondary stroke prevention in subjects with ICAS.

This study will provide preliminary data to enable planning of a subsequent grant submission to NIH to perform a Phase I / II trial with the following goals: to provide more rigorous data on the mechanisms of action of RLIC, and to determine the potential efficacy of RLIC for preventing stroke in high-risk subjects with ICAS. Our ultimate goal is to perform a large randomized multicenter NIH-funded Phase III trial to determine the efficacy of RLIC for preventing stroke.

3. OVERVIEW OF STUDY DESIGN

The design of the study is illustrated in Figure 1 below. In this prospective randomized pilot study, 10 eligible high-risk subjects with ICAS will be randomized to RLIC (bilateral upper extremity (BUE) daily for 30 days) plus AMM (n=5) or AMM alone (n=5). The AMM, which will be started at study enrollment and continued until close-out in all subjects, will consist of aspirin 325 mg per day, clopidogrel 75 mg per day, and risk factor management primarily targeting a systolic BP < 140 mmHg and LDL cholesterol < 70 mg /dl. All subjects will undergo baseline brain arterial spin labeling (ASL) and perfusion MRI to measure CBF and have blood drawn for biomarkers 3-5 days after randomization to allow for washout of any effect from the test RLIC treatment that will be done prior to randomization to determine the subjects' tolerability to RLIC treatment (see section 5 for more details on screening test). The period of 3-5 days will provide flexibility for scheduling these tests. After the baseline MRI and biomarker tests are completed, the subjects randomized to RLIC will begin daily RLIC for 30 days. Each daily BUE RLIC treatment will consist of 4 cycles of 5-minute inflations of both blood pressure cuffs simultaneously to a pressure of 200 mm Hg with 5 minutes of reperfusion between each inflation using the Doctormate device (see Figure 2). All subjects will return for their close-out visits 33-35 days after enrolling in the study and will undergo brain ASL and perfusion MRI and have blood drawn for biomarkers at that visit. The study will be conducted at 4 sites (MUSC, MCG, UCLA, USC) to enable us to evaluate the consistency of CBF measurements across multiple sites and to ensure that the recruitment target of 10 subjects will be met in time to allow for the subsequent NIH grant submission in 2017.

Figure 1. Overview of Study Design



4. SUBJECT SELECTION CRITERIA

The Target Population are subjects between 30 – 90 years of age with stroke within the previous 30 days that is attributed to 70-99% atherosclerotic stenosis of a major intracranial artery (carotid, middle cerebral, vertebral or basilar). The full inclusion and exclusion criteria are similar to the SAMMPRIS trial [8] (referred to in section 1 above) with the following exceptions: 1) Subjects with TIA will be excluded because of their excellent outcome on AMM in SAMMPRIS.[10] 2) The upper age limit in this pilot is 90 rather than 80, given the positive response to RLIC in octogenarians in the 2nd Chinese trial.[17] 3) CT angiography (CTA) or magnetic resonance angiography (MRA) measurements of 70-99% intracranial stenosis will be sufficient for enrollment in this study, whereas catheter angiography was required in SAMMPRIS. (Catheter angiography is expensive and has some risk that is difficult to justify in a medical management trial). 4) Diabetic subjects taking sulfonylureas will be excluded because these drugs block ATP-sensitive potassium channels that are thought to play a key role in the protective effect of RLIC.[18] The full inclusion and exclusion criteria for this pilot study are provided below.

Inclusion Criteria:

1. Symptomatic cerebral infarction within 30 days of enrollment attributed to 70-99% stenosis of a major intracranial artery (carotid artery, MCA stem (M1), vertebral artery, or basilar artery) that is documented by any of the following: MRA, CTA, or catheter angiography. Percent stenosis will be measured according to WASID criteria (= 1 – [Ds / Dn]) x 100% with Ds [diameter of stenosis] and Dn [diameter of normal vessel]).[19]
2. Modified Rankin score of ≤ 3
3. Age ≥ 30 years and ≤ 90 years.
    - Subjects 30-49 years are required to meet at least one additional criteria (i-vi) provided in the table below to qualify for the study. This additional requirement is to increase the likelihood that the symptomatic intracranial stenosis in subjects 30-49 years is atherosclerotic.

i. insulin dependent diabetes for at least 15 years
ii. at least 2 of the following atherosclerotic risk factors: hypertension (BP ≥ 140/90 or on antihypertensive therapy); dyslipidemia (LDL ≥ 130 mg /dl or HDL < 40 mg/dl or fasting triglycerides ≥ 150 mg/dl or on lipid lowering therapy); smoking; non-insulin dependent diabetes or insulin dependent diabetes of less than 15 years duration; family history of any of the following: myocardial infarction, coronary artery bypass, coronary angioplasty or stenting, stroke, carotid endarterectomy or stenting, peripheral vascular surgery in parent or sibling who was < 55 years of age for men or < 65 for women at the time of the event
ii. history of any of the following: myocardial infarction, coronary artery bypass, coronary angioplasty or stenting, carotid endarterectomy or stenting, or peripheral vascular surgery for atherosclerotic disease
iv. any stenosis of an extracranial carotid or vertebral artery, another intracranial artery, subclavian artery, coronary artery, iliac or femoral artery, other lower or upper extremity artery, mesenteric artery, or renal artery that was documented by non-invasive vascular imaging or catheter angiography and is considered atherosclerotic
v. aortic arch atheroma documented by non-invasive vascular imaging or catheter angiography
vi. any aortic aneurysm documented by non-invasive vascular imaging or catheter angiography that is considered atherosclerotic

4. Negative pregnancy test in a female who has had any menses in the last 18 months
5. Subject is willing and able to return in 30 days for close-out visit for the study
6. Subject is available by phone
7. Subject is able to apply the conditioning device or has access to another person (family member, friend) who can assist with application of conditioning device if needed
8. Subject understands the purpose and requirements of the study, can make him/herself understood, and has provided informed consent
9. Subject is able to undergo brain MRI

Exclusion Criteria:

1. Previous treatment of target lesion with a stent, angioplasty, or other mechanical device, or plan to perform one of these procedures
2. Plan to perform concomitant angioplasty or stenting of an extracranial vessel tandem to an intracranial stenosis
3. Intracranial tumor (except meningioma) or any intracranial vascular malformation
4. Thrombolytic therapy within 72 hours prior to enrollment
5. Progressive neurological signs within 24 hours prior to enrollment
6. Any intracranial hemorrhage (parenchmal, subarachnoid, subdural, epidural) within 90 days
7. Any untreated chronic subdural hematoma

8. Intracranial arterial stenosis due to arterial dissection, Moya Moya disease; any known vasculitic disease; herpes zoster, varicella zoster or other viral vasculopathy; neurosyphilis; any other intracranial infection; any intracranial stenosis associated with CSF pleocytosis; radiation induced vasculopathy; fibromuscular dysplasia; sickle cell disease; neurofibromatosis; benign angiopathy of central nervous system; post-partum angiopathy; suspected vasospastic process, suspected recanalized embolus
9. Presence of any of the following unequivocal cardiac sources of embolism: chronic or paroxysmal atrial fibrillation, mitral stenosis, mechanical valve, endocarditis, intracardiac clot or vegetation, myocardial infarction within three months, dilated cardiomyopathy, left atrial spontaneous echo contrast, ejection fraction less than 30%
10. History of upper extremity ischemia, known subclavian or brachial artery stenosis, subclavian steal syndrome, any upper extremity soft tissue, orthopedic or vascular injury, or mastectomy or other procedure that may contraindicate taking blood pressure or having a cuff on the arm for the conditioning treatment
11. Difference in systolic blood pressure of > 15 mm Hg between both arms
12. Known allergy or contraindication to aspirin or clopidogrel
13. Active peptic ulcer disease, major systemic hemorrhage within 30 days, active bleeding diathesis, platelets < 100,000, hematocrit < 30, INR > 1.5, clotting factor abnormality that increases the risk of bleeding, current alcohol or substance abuse, uncontrolled severe hypertension (systolic pressure > 180 mm Hg or diastolic pressure > 115 mm Hg), severe liver impairment (AST or ALT > 3 x normal, cirrhosis), subject on dialysis
14. Major surgery (including open femoral, aortic, or carotid surgery, cardiac) within previous 30 days or planned in the next 30 days after enrollment
15. Indication for warfarin or heparin beyond enrollment (**NOTE: exceptions allowed for subcutaneous heparin for deep vein thrombosis (DVT) prophylaxis while hospitalized**)
16. Diabetic subjects taking sulfonylurea drugs
17. Severe neurological deficit that renders the subject incapable of living independently
18. Dementia or psychiatric problem that prevents the subject from following the protocol reliably
19. Co-morbid conditions that may limit survival to less than 3 months
20. Pregnancy or of childbearing potential and unwilling to use contraception for the duration of this study
21. Claustrophobia requiring sedation for MRI or any metallic implants or MRI incompatible devices that do not permit the patient to have an MRI
22. Enrollment in another study that would conflict with the current study

5. SCREENING, INFORMED CONSENT, RANDOMIZATION AND ENROLLMENT OF SUBJECTS

Subjects will be screened from the inpatient and outpatient stroke service at all four participating sites. All eligibility criteria are determined by evaluations performed as routine standard of care. The principles of Informed Consent, according to FDA Regulations and ICH guidelines on GCP, will be followed. Each Investigator will submit a copy of the proposed consent form, together with the study protocol, to the appropriate IRB for approval. All subjects must provide informed consent to participate and only the participant can provide informed consent, which must be cosigned by a study investigator. Once a subject's eligibility has been confirmed by the site investigators, the subject will be approached to participate in the trial. The consent form includes a section describing a screening process whereby the subject will have a test RLIC treatment with the Doctormate device (Figure 2) with inflation of the blood pressure cuffs on both arms simultaneously to a SBP of 200 mmHg for 5 minutes to ensure that the subject can tolerate the treatment. Subjects who cannot tolerate the treatment will be called by the study coordinator at 1 week but will not participate further in the trial. If the subject can tolerate the screening treatment and wishes to participate in the trial, the coordinator will email the study statistician at MUSC who will provide the randomized treatment assignment by email.

6. RLIC DEVICE AND ISCHEMIC CONDITIONING REGIMEN

The RLIC device in this pilot (called Doctormate) will be donated by the company that provided the device for the Chinese pilot studies. Dr. David Hess (co-investigator in this study) obtained an amendment to an existing IDE approval from the FDA (G140239) to use this device in a small IRB-approved pilot study at MCG and

MUSC in late 2015. The purpose of that pilot study was to obtain initial experience with the Doctormate device. In that study, 4 subjects with symptomatic ICAS (1 at MUSC, 3 at MCG) were treated with daily BUE RLIC (4 cycles of 5-minute inflations to a pressure of 200 mm Hg with 5 minutes of reperfusion between each inflation) for 30 days to assess their tolerance of the procedure. CBF and biomarkers were not performed. The subject at MUSC performed RLIC daily for 7 days and tolerated each treatment well but then developed unilateral wrist pain (unrelated to active treatment). Subsequent measurements of blood pressure and pulses in both arms were similar. The subject was willing to continue treatment but, because this was a preliminary feasibility study, Dr. Chimowitz decided not to continue the subject's treatment to 30 days. The 3 subjects enrolled at MCG tolerated the treatment well with 30-day treatment adherence rates of 100%, 90% and 85%. Drs. Chimowitz and Hess have also personally used the RLIC device daily for 14 days (see Figure 2) and were easily able to tolerate the mild symptoms produced by the treatment (paresthesia, mild cyanosis of fingers). Dr. Hess has now applied to the FDA to extend the IDE approval to cover the pilot study proposed in this protocol.

Figure 2. The RLIC device used in the Chinese trials will be used in this pilot study and is shown being applied to Dr. David Hess, a Co-PI of this pilot study at Georgia Regents University (center)

Rationale for the RLIC regimen in this study. Each daily bilateral upper extremity RLIC treatment will consist of 4 cycles of 5-minute inflations of both blood pressure cuffs simultaneously to a pressure of 200 mm Hg with 5 minutes of reperfusion between each inflation using the Doctormate device (see Figure 2). The selection of 4 cycles of 5-minute duration for the daily RLIC treatment is based on the initial ischemic preconditioning study in canine hearts by Murry in 1986 that established the effectiveness of this regimen.[20] Moreover, in terms of acute cardioprotection, pre-clinical studies have shown that 4 to 6 cycles of 5-minute durations appear optimal.[21] Using 4 cycles instead of 5 cycles (the regimen used in the Chinese trials [16,17]) will lessen the total duration of each treatment from 45 minutes to 35 minutes, which we expect will increase adherence. We chose to measure changes in CBF and biomarkers at 30 days for this pilot study because some preliminary human studies by the MCG group show that CBF increases within 6-24 hours of first use of RLIC, and a preclinical study (in a mouse model) shows that the increased CBF after RLIC is sustained to 28 days with daily RLIC treatment.[12] Moreover, the first 30 days after enrollment in SAMMPRIS was the highest risk period for recurrent stroke so it is important to determine if the CBF and biomarker responses to RLIC persist for this period.

7. AGGRESSIVE MEDICAL MANAGEMENT

The medical management for all subjects enrolled in this pilot study will consist of the current standard of care recommended by the American Heart Association and American Stroke Association for patients with ICAS established by the SAMMPRIS trial.[22] This consists of dual antiplatelet therapy (aspirin 325 mg per day, clopidogrel 75 mg per day) and intensive risk factor management targeting a systolic blood pressure < 140 mm HG, an LDL cholesterol < 70 mg /dl, non-HDL cholesterol < 100mg/dl, smoking cessation, and hemoglobin A1c < 7% in diabetics.

8. BLOOD BIOMARKER MEASUREMENTS

The blood biomarkers that will be measured are putative biomarkers of RLIC [23-27] and known inflammatory and fibrinolytic markers associated with the development or progression of ICAS, or increased risk of stroke in patients with ICAS [28-31]. These consist of: Nitrite, IL-10, SDF1, Micro RNA 144, Lp-PLA2, IL-6, hsCRP, Soluble E-selectin, ICAM, MMP-9, PAI-1, tPA, and ADMA. We will measure the blood biomarkers at baseline and close-out (33-35 days after enrollment) in all 10 subjects. All blood biomarkers will be mailed to the Blood Biomarker Core at MCG where the levels of biomarkers will be measured blinded to site, subject, treatment assignment and time point.

9. MRI MEASUREMENTS OF CBF AND OTHER PERFUSION PARAMETERS

ASL MRI will be used to provide quantitative measurements of CBF in all 10 subjects in the study (at baseline and at close-out). [32,33] ASL does not require the injection of contrast material and can be repeated over time without risk to patients. **We will use a standardized ASL protocol agreed upon by the study radiologists and physicists at the 4 participating sites that can be applied to different MRI scanner platforms**.[34] Each subject will undergo all MR studies for this pilot on the same scanner to limit slights variations in images from scanner to scanner. We have already tested the feasibility of using our planned ASL MRI protocol under a MCG / UCLA IRB-approved protocol. Six subjects with recent stroke from ICAS underwent ASL MRI before and after one RLIC treatment, a mean of 4.6 hours apart. The de-identified scans were sent to UCLA where Dr. Liebeskind performed CBF analyses and readings blinded to pre- and post-RLIC treatment. After just 1 cycle of RLIC, these subjects showed mean increases of CBF of 8%-28% in the MCA and PCA territories on the infarct side vs. the normal side. While quantitative CBF obtained with ASL MRI is the primary MR outcome in this pilot, we will also utilize dynamic susceptibility contrast enhanced perfusion MR imaging (DSC-PWI) to assess the effectiveness of secondary brain perfusion parameters (such as cerebral blood volume and cerebrovascular transit times).

10. ASSESSMENT OF FEASIBILITY, TOLERABILITY AND ADHERENCE TO RLIC

Enrolled subjects randomized to the RLIC group will take the Doctormate device home and use it daily for the next 30 days. The research coordinator will call the subject weekly up until the close-out visit to document any problems with the device or any adverse events using a questionnaire (see Appendix 1). The subject will also be asked to keep a daily diary for the 30 days the Doctormate device is used (see appendix 2.) The device records when it is used and whether the treatment was completed so when the subject returns for the close-out visit, we will be able assess adherence to use of the device.

11. RANDOMIZATION, BLINDING, AND DATA MANAGEMENT

Subjects will be randomized 1:1 using a pre-generated exact central randomization list that controls for moderate imbalances at the site level. All study data will be entered directly by either the study PI or stroke coordinator at each site in the RedCap data management system developed at MUSC for the study. Initial data checking will be performed by the system before the data can be saved to prevent low level data errors. When the data are transferred to the study biostatistician for analysis and report generation, further data quality checks will be conducted by SAS. Study data will be retained on a secure server at the Department of Public Health Sciences at MUSC.

12. STATISTICAL ANALYSES

To estimate the variability in CBF and biomarkers, all of which are continuous measures, simple summary statistics will be reported for the baseline and close-out measurements in each group. With five imaging samples per group, the precision of the 90% confidence interval around the mean would be ± 0.95 for a SD of 1, and, as an example, would increase to ± 7.63 for a SD of 8. We will also evaluate the changes in CBF and biomarkers from baseline to close-out in the two groups using means and 90% confidence intervals. Here, the precision of the resulting confidence interval is ± 9.4 when the SD is 8; that is, if RLIC yields a mean 10% change in CBF and sham RLIC yields a mean 0% change in CBF the resulting 90% confidence interval is (0.6, 19.4) for the difference in percent change in CBF. Finally, with a sample size of N=5, we will be able to estimate the correlation between CBF and biomarkers for each arm with an 90% confidence interval of 0.5 (-0.5, 0.9) in the case of an observed moderate correlation. Interpretation of the summary statistics will be complimented with graphical approaches including boxplots to assess distributions within treatment arms, and funnel plots to assess differences at the site level.

13. PROTECTION OF HUMAN SUBJECTS

Risks to the Subjects: The risks of the Doctormate device might include discomfort, numbness or tingling in the hands and arms when the device is inflated, and a warm feeling when it is deflated. The device may also cause bruising in the arm or petechiae. There is also the theoretical possibility of a small risk of a venous clot

forming in the arm. However, in our review of the literature we have not found any reports of blood clot formation with RLIC and there were no instances of venous thrombosis or any serious adverse event in either of the two published trials in China. [16,17] In one of those trials, 30 subjects over age 80 used the Doctormate device twice per day on both arms for 6 months and the following risks were noted in that trial: "Transient sporadic petechiae were observed in 3 cases during the first 30 days of the study, but disappeared soon after without discontinuing treatment. No ecchymosis, tenderness to palpation, edema, skin breakage, or other skin lesions were observed. No deep vein thromboses were detected by vascular sonography during or at the end of the 180 days of treatment".[17] In our pilot trial, we will only be using the device once per day for a period of 30 days, which is a considerably lower dose than previously used in the two Chinese trials. In both animal and human experiments for cardiac or brain conditioning, there are no examples where RLIC increased CBF above normal levels or resulted in intracerebral hemorrhage.

Risk Analysis and Risk Mitigation

All SAEs and any deaths will be reported to the Institutional Review Board within 24 hours.

The risks related to participation in this trial are related to the a) RLIC device; b) MRI scan; c) phlebotomy; d.) medications associated with AMM; and e) loss of confidentiality.

a.) Device related. Although we don't anticipate any serious adverse events related to the RLIC device based on experience with the same device in two Chinese trials [16,17] , patient safety is of paramount importance in this trial and there are operations in place for monitoring adverse events (see Protection Against Risk below). The risks that are possibly anticipated with the RLIC device include:
- Petechiae on the arm
- Skin rash or hypersensitivity to the cuff
- Thrombosis of veins in arm
- Swelling of the arm
- Pain and discomfort in the arm
- Muscle or nerve injury in the arm

b.) The risks that are associate with MRI include
- Claustrophobia and anxiety from the MRI

c.) The risks associated with phlebotomy are
- Discomfort or bruising of an arm from venipuncture required for biomarkers and routine blood tests

d.) All of the medications used in this study (aspirin and clopidogrel and the medications to control risk factors) have been approved by the Food and Drug Administration (FDA) for the conditions for which they will be prescribed in this study and are not investigational. The major side effects of aspirin are major hemorrhage and peptic ulceration. Other possible side effects of aspirin include heartburn, ringing in the ears, nausea and vomiting. The most common side effects of clopidogrel are skin rash, bruising, itching, nausea, vomiting, diarrhea, intestinal discomfort. A less common but more serious side effect of clopidogrel is major gastrointestinal or brain hemorrhage. The most serious side effect of clopidogrel is a clotting disorder called thrombotic thrombocytopenic purpura (TTP) but this is very rare (1 in 15,000-20,000 treated subjects). The combination of clopidogrel and aspirin is commonly used for 90 days after stroke or TIA in patients with intracranial stenosis following the SAMMPRIS trial [9] and will be prescribed for the duration that subjects participate in this pilot study (33-35 days). The combination of aspirin and clopidogrel is associated with a significantly higher risk of major bleeding compared with clopidogrel or aspirin alone. The risks associated with statin therapy are a persistent increase in liver enzymes (0.0 - 0.4%), myalgia (2.8%), muscle weakness with increase in CK levels > 10 x upper limit of normal (0.03 - 0.1%), and rarely rhabdomyolysis that can potentially lead to renal failure. The risks associated with antihypertensive medications include hypotension, dizziness, headaches, weakness,

allergic reactions, diarrhea, hypokalemia or hyperkalemia depending on the type of blood pressure medication taken, cough (with ACE inhibitors), and angioedema (ACE inhibitors).

e.) There is the risk of loss of confidentiality

The principles of Informed Consent, according to FDA Regulations and ICH guidelines on GCP, will be followed. All subjects must provide informed consent to participate and only the participant can provide informed consent, which must be cosigned by a study investigator. Once a subject's eligibility has been confirmed by the site investigators, the subject will be approached to participate in the trial. The consent form includes a section describing a screening process whereby the subject will receive one session of RLIC treatment lasting 5 minutes to ensure that the subject can tolerate the treatment. Subjects who cannot tolerate the treatment will be called by the study coordinator at 1 week but will not participate further in the trial. Only subjects who can tolerate the screening treatment will be randomized to one of the treatment arms.

Protection Against Risk

The research coordinator will call subjects randomized to the RLIC group weekly and will use a standardized questionnaire to ask subjects about adverse events (see Appendix 1). For the pain question, we will utilize a visual analog pain scale of 0 to 10 and provide this to the subjects randomized to the RLIC group. At the weekly phone call, the research coordinator will ask the degree of pain using this scale. If the score is > 2, the site investigator will be notified and will contact the subject by phone to review the level of discomfort. Additionally, If the subject answers "yes" to any other questions, we will ask the subject to come in to be examined. If we suspect a venous thrombosis because of swelling or pain, we will perform a venous ultrasound to rule out a deep venous thrombosis. We will also obtain the phone number of a relative or friend in order to reach the subject if we have trouble reaching the subject. All subjects will be given the telephone numbers of the principal site neurologist and site coordinator to report any clinical events or to ask questions about the study.

Subjects who are claustrophobic will not be enrolled in this study. To reduce the risk of phlebotomy, only trained phlebotomists will perform venipuncture. All neuroimaging and biomarker data will be de-identified as stated under "Sources of Material". Copies of the source documents used to collect data for study subjects will be kept in confidential binders at the participating sites. All these measures will ensure against loss of confidentiality.

Side effects of any medications prescribed to subjects as part of routine care of subjects with intracranial stenosis will also be monitored. These side effects will be minimized by recommending use of enteric-coated aspirin, checking liver enzymes and CK for subjects on a statin if clinically indicated, and instituting appropriate treatment should side effects arise.

Potential Benefits of the Proposed Research to the Subjects and Others. Subjects enrolled in this study may benefit from the increased medical supervision that occurs by participating in this study; however, no benefit from this supervision or the RLIC treatment can be guaranteed. If RLIC is indeed effective as suggested in the previous Chinese trials, [16,17] the subjects randomized to RLIC may have a reduction in stroke. Based on previous human studies, there are minimal risks to RLIC [16,17] and there is the potential that this treatment could ultimately be shown to be effective for lowering the risk of stroke in patients with ICAS, one of the highest risk stroke-prone conditions.

Importance of the Knowledge to be Gained. The risk of recurrent stroke in patients with ICAS is very high, about 20% at one year even with aggressive medical management (AMM). [10] RLIC has the potential to reduce this risk of stroke and avoid disability for patients with ICAS based on the preliminary trials performed in China. [16,17] Since the anticipated risks of RLIC are low, these risks are reasonable in relation to the importance of the knowledge that will be gained from this study. This study will provide key pilot data to evaluate the tolerability and adherence to RLIC and will enable calculation of the sample size for a subsequent larger NIH-funded

multicenter Phase I / II trial that will provide more rigorous data on the mechanisms of action of RLIC and the potential efficacy of RLIC in preventing stroke in these subjects. If these two goals are achieved in the Phase I / II trial, we will submit another grant to NIH to fund a definitive large multicenter Phase III trial to establish the efficacy of RLIC for subjects with ICAS. If that planned trial is positive for RLIC, it will lead to a new treatment for this common and high-risk disease and will provide a strong rationale to test RLIC for related conditions such as patients with extracranial carotid stenosis and patients with vascular cognitive impairment

Safety Monitoring. Fenwick Nichols MD, Professor of Neurology at the Medical College of Georgia, will serve as the Safety Officer. Dr. Nichols has over 30 years of experience as a vascular neurologist, is Board Certified in Internal Medicine and Neurology, and served as the Safety Officer for a past trial of minocycline in acute ischemic stroke conducted at the Medical College of Georgia, and two other sites. All adverse events (AE) and serious adverse events (SAEs) will be reported to Dr. Nichols and the site's IRB within 24 hours. A SAE is defined by any medical experience regardless of its relationship to the investigational device or study drugs that occurs during subject enrollment in this trial that results in any of the following: (1) inpatient hospitalization or prolongation of a hospitalization; (b) persistent or significant disability/incapacity; (c) death of the study subject, or (d) necessitates an intervention to prevent a permanent impairment of a body function or permanent damage to a body structure. Dr. Nichols will make a determination of whether an AE or SAE in any way is related to the Doctormate device.

Stopping rules: If the Safety Officer determines that an SAE is definitely related to the device, the study will be stopped and a review will be conducted by the safety monitor of the outcomes of all enrolled subjects, who will determine whether enrollment in the study can be restarted. Since there are only 10 subjects that will be enrolled in this pilot study, this decision to restart enrollment cannot be based on a formal statistical stopping rule. Instead it will be based on a clinical impression of likely harm to subjects from continuing ischemic conditioning, which the evidence from much larger completed trials of ischemic conditioning in subjects with coronary and intracranial occlusive disease suggests is very unlikely. [16,17,35]

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Appendix 1. **Adverse Event Questionnaire**

Subject Study Number _____

End of Week (Circle one) 1._____, 2._____, 3. _____, 4. (close-out visit) _____

Date_____

1.) Have you experienced any swelling in either of your arms? *(Y/N)*
Coordinator Comment: (if yes, specify which arm(s) and where in the arm(s), i.e., above, at or below cuff)

2.) Have you had any discoloration (change in color) in either of your arms after any treatment? *(Y/N)*
This could be redness, blueness, small red dots, red or purple blotches in your arm
Coordinator Comment: (*if yes, specify which arm(s), where in the arm(s), and type of discoloration)*

3.) Have you had any persistent tingling or numbness in either of your arms? *(Y/N)*
Coordinator Comment: (if yes, specify which arm(s) and where in the arm(s))

4.) During the cuff inflations or any time between use of the cuff, have you had pain in either arm? *(Y/N*)
Coordinator Comment: if yes, ask subject to use visual analog pain scale given to subject after enrollment to rate the discomfort on the Pain scale between 0 and 10, and specify which arm(s) and where in the arm(s)is the pain located) Pain rating_____

Appendix 2. **Subject Diary**

Subject Study Number _____

Day	Completed? Y/N	Pain in an Arm? Y/N Grade (0-10) If yes, call coordinator	Arm discoloration, swelling, or tingling? (Bruising, etc). Y/N If yes, call coordinator	Specify type of problem or any other comments
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Research Coordinator Name and Contact Number:

Principal Investigator Name and Contact Number: