Remote Ischemic Preconditioning Over Time to Empower Cerebral Tissue (REM-PROTECT)

NCT: 02169739

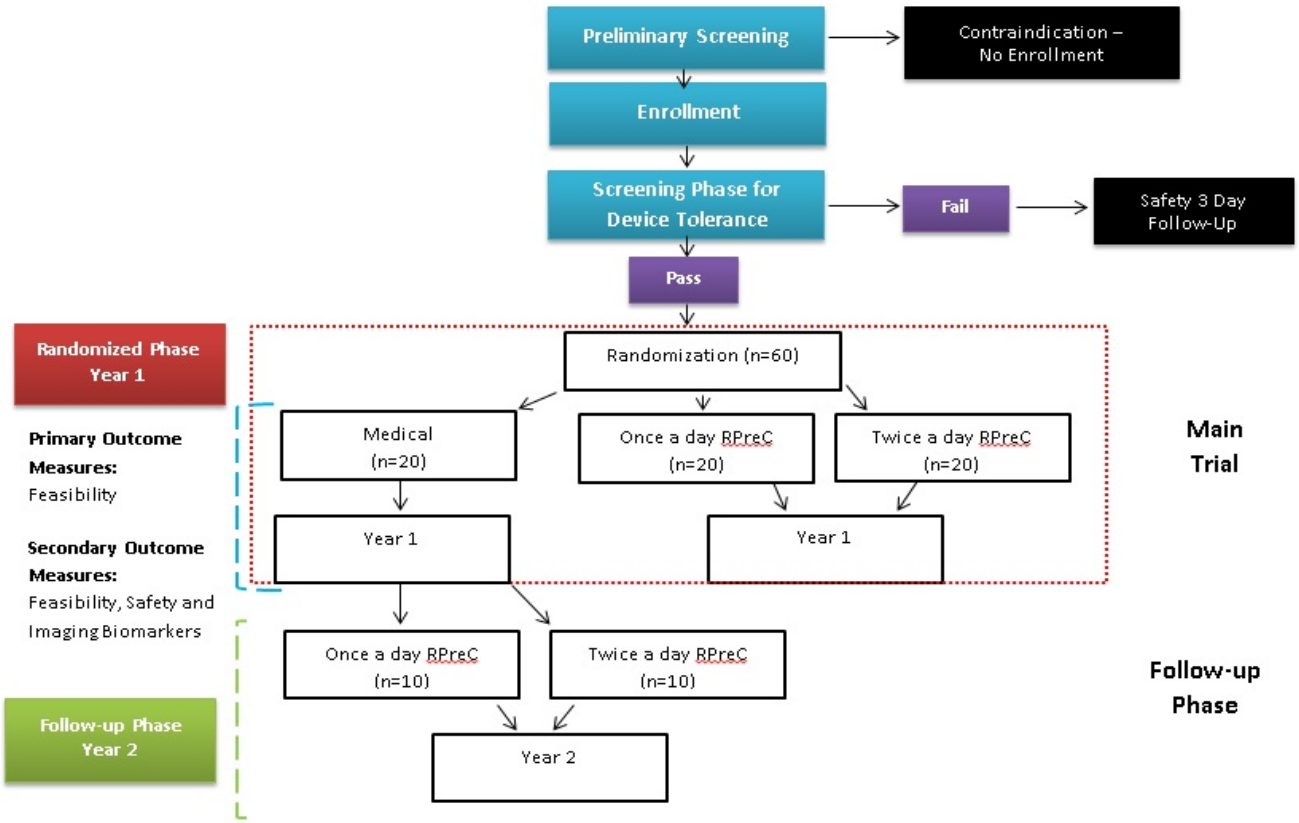
March 8th, 2016

SUMMARY OF CHANGES DOCUMENT

PROTOCOL:
Remote Preconditioning Over Time to Empower Cerebral Tissue (REM-PROTECT) Clinical Trial

RATIONALE FOR CHANGES	
1.	**Added Screening Phase for Device Tolerance** After a subject consents to participate in the study, he/she will first participate in a study screening phase to ensure a basic level of tolerability of Remote Ischemic Conditioning (autoRIC™) device. The subject will undergo one full cycle of treatment under observation of the study team, including 4 cycles of alternating 5 minute inflation and 5 minute off periods. If the subject indicates willingness to continue receiving such treatment (screening success), she/she will enter the randomized trial phase, and be randomly allocated to the treatment or control group. If subject indicates unwillingness to continue receiving such treatment (screening failure), he/she will not advance to the randomized phase of the trial. Screen failure subjects will be followed up with a 3 day post-device screening phone call to ensure safety and obtain information regarding any adverse events.
2.	**Changed Figure 5. Flow Diagram of the Study Design.** This change reflects new changes in the clinical protocol (see below)
3.	**Device Compliance added by Manufacturer.**

REM-PROTECT REMOTE ISCHEMIC PRECONDITIONING OVER TIME TO EMPOWER CEREBRAL TISSUE



PROTOCOL:
Remote Preconditioning Over Time to Empower Cerebral Tissue (REM-PROTECT) Clinical Trial

Version: B

Date: March 8, 2016

Overview of Protocol of REM-PROTECT

Title	REMote Preconditioning Over Time to Empower Cerebral Tissue (REM-PROTECT)
Objective	To evaluate remote limb ischemic preconditioning (RPreC) as a novel therapeutic strategy to prevent stroke, progressive ischemic brain injury, and cognitive decline in patients with moderate to severe cerebral small vessel ischemic disease.
Study Design	A single site, feasibility, dose-ranging randomized, medication-device group
Population Studied	60 patients referred or seen at the UCLA clinic Inclusion criteria: 1) likely a lacunar stroke syndrome within the past 6 months, 2) age 40-above, 3) absence of signs or symptoms of cortical dysfunction, 4)MRI presence of a small subcortical ischemic, 5) white matter hyperintensity score of 2 (moderate) or 3 (severe) on the European Scale of Age-Related White Matter Change.
	Randomized Phase: Medical (control) or Remote Ischemic PreConditioning (RPreC)
Device	Automated Remote Ischemic Conditioning (autoRIC™) device and cuffs
Number of Subject Enrolled	3
Number of Devices Shipped	6

A. Specific Aims

The general aim of Remote Preconditioning Over Time to Empower Cerebral Tissue (REM-PROTECT) is to evaluate remote limb ischemic preconditioning (RPreC) as a novel therapeutic strategy to prevent stroke, progressive ischemic brain injury, and cognitive decline in patients with moderate to severe cerebral small vessel ischemic disease. A single site, feasibility dose-ranging randomized clinical trial of RPreC will be performed.

This is a feasibility early phase device trial to determine the feasibility of therapy adherence during chronic use before going to larger trial. Long-term compliance with this treatment with some nuisance aspects in a US small vessel stroke population is unknown and this fundamental piece of

information is needed to plan any subsequent trial. We want to test this technology and hope to achieve a goal of at least 75% or more compliance for physiologic targets.

During periods of active treatment, RPreC will be induced twice a day by using the autoRIC™ device (which delivers four remote ischemic conditioning cycles of five minute intervals followed by five-minutes of normal blood flow) around both upper body extremities. The RPreC intervention will be add-on therapy to guideline-based best standard medical prevention therapy. The study will have a randomized phase and a follow-up phase. 60 enrolled patients will be randomized 2:1 to best standard medical care plus active RPreC for 1 year and then follow-up for 1 year versus best standard medical care alone for 1 year and then active RPreC for 1 year. Primary outcome measures will be obtained at the end of the first randomized year (staggered start RCT design). Successful completion of this trial will delineate the feasibility and safety, and explore biomarker indicators of potential efficacy, of inducing brain ischemic tolerance in small vessel stroke-prone patients.

A.1. Specific Aim 1: Feasibility

To establish the feasibility of remote ischemic preconditioning as a sustained therapy for prevention of ischemic brain injury from cerebral small vessel disease.

This aim will be accomplished by demonstrating the remote ischemic preconditioning treatment can be successfully implemented with more than 75% adherence among enrolled patients throughout the 1-year of active treatment. Both patient self-report and remote physiologic monitoring will be performed to quantify patient behavioral adherence to the treatment regimen, and remote physiologic monitoring will be used to quantify physiologic attainment of brief ischemia states in the upper extremities. Establishment of feasibility will allow for subsequent larger, definitive clinical trials of RPreC as a treatment to confer long-term protection against ischemic brain injury due to cerebral small vessel disease.

A.2. Specific Aim 2: Safety

To determine the safety of remote ischemic preconditioning as a sustained therapy for prevention of ischemic brain injury from cerebral small vessel disease.

This aim will be accomplished by assessing safety outcomes in patients undergoing remote ischemic preconditioning treatment. The primary safety endpoint will be all serious adverse events. Secondary safety endpoints will include deep and superficial vein thrombosis and all-cause mortality. Ascertainment that RPreC poses no major excess risk to patients will allow for subsequent efficacy testing in a larger clinical trial.

A.3. Specific Aim 3: Signals of Efficacy

To employ imaging biomarkers to demonstrate biologic efficacy of remote ischemic preconditioning as a sustained therapy for prevention of ischemic brain injury from cerebral small vessel disease, and to employ cognitive, ambulation, and clinical stroke event outcome measures to explore potential clinical efficacy.

This aim will be accomplished by measuring volumetric progression of white matter ischemic injury on magnetic resonance imaging (biomarker efficacy) and cognitive battery performance,

ecologic ambulation assessment, and recurrent stroke events (clinical efficacy). MRIs will be performed at baseline and at 1 year (end of randomized phase) and several markers of injury progression assessed, including FLAIR hyperintensity volumetric burden (primary biomarker efficacy outcome), diffusion tensor imaging volumetric burden, and cerebral atrophy. Wireless sensors will be used to assess gait quantity and quality in real-world activity at baseline and 1 year. A cognitive battery sensitive to executive, attentional, and memory function, reflecting subcortical-frontal system integrity, will assess progression of vascular cognitive impairment. Lastly, clinical events during the year of randomized therapy will be compared across treatment arms, including ischemic stroke, transient ischemic attack, hemorrhagic stroke, myocardial infarction, and cardiovascular death.

C. Trial Design

C.1. Study Overview

This is a single site, feasibility, randomized, dose-ranging clinical trial of remote ischemic preconditioning. During periods of active treatment, induced twice a day by using the autoRIC™ device around both upper body extremities which would deliver four remote ischemic conditioning cycles of five minute intervals followed by five-minutes of normal blood flow. The RPreC intervention will be add-on therapy to guideline-based best standard medical prevention therapy. The study will have a randomized phase and a follow-up phase. 60 enrolled patients will be randomized 1:1:1 to 1 year of best standard medical care alone, best standard medical plus active once-daily RPreC, or best standard therapy plus active twice-daily RPreC. Following the 1 year randomized phase, the medical control group will cross over to once (10 patients) and twice (10 patients) daily RPreC for 1 additional year (staggered start design, allowing for collection of additional feasibility and safety data and control group adherence to follow-up). Primary outcome measures will be obtained at the end of the first randomized year.

C.2. Technology

Remote Ischemic Preconditioning Device: The autoRIC™ device is a new, portable, automated conditioning device, designed and developed by CellAegis Devices Inc., Canada. The device consists of a Control Unit, Charging Cradle, a Power Adaptor, a Wall Mount for the charging cradle (with wall plug and screws) and Applicator Cuffs. The control unit is a stand-alone reusable controller that controls the inflation and deflation cycles of the Applicator Cuff. The Control Unit clicks into the disposable Applicator Cuff when it is ready for use. The Applicator Cuff is a single-used, disposable cuff that is fastened using Velcro into which a 'one button' battery-driven actuator system inserts. This provides for a mobile 'point-of-care' delivery of RIC that will not impose on other care delivery, and requires no special expertise for its use. The active device is programmed to inflate the cuff to 200 mmHg for five (5) minutes (ischemia) then be deflated for five (5) minutes (reperfusion) completing one cycle of ischemia-reperfusion; the RIC therapy cycle consists of a total of four (4) cycles of ischemia-reperfusion, which the autoRIC Device is programmed to automatically deliver.

In this study, the device will be used with Applicator Cuffs bilaterally for 12 months to provide ischemic preconditioning therapy to the patient's upper extremities. The applicator cuffs are available in three different adult sizes according to the SP10 standard (small, medium or large) and the cuff is selected to match the arm size of the patient. To assure a total cessation of blood flow

through the limb during the ischemic duration, the cuff inflation pressure is maintained at 200 mm Hg. By Doppler ultrasound, it has been documented that inflation of the blood pressure cuff to 200mm Hg causes cessation of blood flow in the arm[1, 2]. A total of four (4) inflation and deflation cycles will be applied in five (5) minute intervals twice a day. At the end of this automated process, the Procedure Complete indicator is illuminated and an audible tone is heard. The autoRIC™ will be used for 12 months to deliver remote ischemic conditioning to the patient. There have been no reported experiences of any side effects in preclinical and clinical studies.

In addition to the autoRIC® Device, CellAegis Devices, Inc. developed the CRICtrac™ Mobile Application that allows the clinician and coordinators to monitor compliance of each individual study participant. Once the patient has been assigned to a treatment group, the coordinator will initialize new patient cuffs by scanning them using the CRICtrac™ Mobile App on a compatible Android smartphone equipped with a near-field antenna utilizing a Wi-Fi network connection. During the course of the trial or at the end of the trial, patient compliance can be monitored using the CRICtrac™ Data Portal providing that the cuff has been scanned. The compliance data is immediately uploaded to the CRICtrac™ Data Portal. The Data Portal can then be accessed via a link from the Mobile App or on any device with internet access. Once logged in, Trial Coordinators can view and/or export patient and trial information.

C2.1. Device Training

All participating investigators and site personnel that will be performing the autoRIC procedure will be trained in the use of the study device prior to participating in the study. Device training will be conducted by the Principal Investigator. Training will be done according to the autoRIC User Manual and will include instructions on charging the device prior to the first use, recharging the device after each procedure, verifying the device is charged prior to each procedure, recognizing and addressing the different Warning/Error indications (e.g., battery light flashing blue, indicating that the battery is low but can still perform at least one procedure, battery light steady red, indicating battery low error – not enough battery power to run a complete procedure, etc.) and specific instructions for returning the device to the sponsor in the event of an error notification that cannot be addressed by the site personnel, or a failure of the device to charge after three hours.

All device training will be documented in a training log that will be maintained in the site regulatory binder.

C.3. Subjects

60 subjects will be identified prior to discharge from the inpatient Stroke Service, which manages over 350 patients yearly, and from the inpatient rehabilitation unit that cares for 100 patients yearly with disabling stroke. They will also be identified in our outpatient stroke clinic that manages over 500 patients yearly. Based upon a review of the past 5 years of stroke patients evaluated, we anticipate that at least 20% are likely to be eligible. Potential participants will be approached by an investigator and given the informed consent from to review. Enrolled subjects will be randomized 2:1 to active treatment (40 patients) and control (20 patients).

C.3.1.Entry Criteria

To enter the study screening phase, patients must meet all of the following inclusion and exclusion criteria:

Inclusion Criteria

I. Clinical

1. Clinical lacunar stroke syndrome within the past 6 months (from among the following 12 syndromes as per SPS3 criteria: pure motor hemiparesis (PMH), pure sensory stroke, sensorimotor stroke, ataxic hemiparesis, dysarthria-clumsy hand syndrome, hemiballism, PMH with facial sparing, PMH with horizontal gaze palsy, PMH with contralateral III palsy, PMH with contralateral VI palsy, cerebellar ataxia with contralateral III palsy, pure dysarthria)
2. Absence of signs or symptoms of cortical dysfunction
3. No proximal large vessel atherosclerosis, intracranial atherosclerosis or cerebellar stroke.
4. No major cardioembolic source requiring anticoagulation or other specific therapy

II. Imaging

1. MRI presence of a small subcortical ischemic per SPS criteria, any 1 or more of:
    a. Diffusion-weighted imaging lesion < 2.0cm in size at largest dimension and corresponding to the clinical syndrome.
    b. Well delineated focal hyperintensity <2.0 cm in size at largest dimension (including rostro-caudal extent) on FLAIR or T2 and clearly corresponding to the clinical syndrome. If other focal hyperintensities are present, the case will be discussed with the PI prior to randomization
    c. Multiple (at least 2) hypointense lesions of size 0.3-1.5 cm at largest dimension (including rostro-caudal extent) only in the cerebral hemispheres on FLAIR or T1 in patients whose qualifying event is clinically hemispheric
    d. Well delinated hypointense lesion <1.5 cm in size at the largest dimension (including rostro-caudal extent) on FLAIR or T1 corresponding to the clinical syndrome. MRI must be done at least 1 months after the qualifying stroke
2. Absence of cortical stroke and large (> 1.5cm) subcortical stroke, recent or remote
3. White matter hyperintensity score of 2 (moderate) or 3 (severe) on the European Scale of Age-Related White Matter Change
4. No evidence of cerebral amyloid angiopathy as per Boston Criteria.

Exclusion Criteria

To be eligible for entry into the study, none of the following criteria may be present:

1. Disabling stroke (Rankin Scale ≥4)
2. Previous intracranial hemorrhage (excluding traumatic) or hemorrhagic stroke
3. Age under 40 years
4. High risk of bleeding (e.g. recurrent GI or GU bleeding, active peptic ulcer disease, etc.)
5. Anticipated requirement for long term use of anticoagulants (e.g. recurrent DVT)
6. Prior cortical or retinal stroke (diagnosed either clinically or by neuroimaging), or other prior cortical or retinal TIA
7. Prior ipsilateral carotid endarterectomy
8. Impaired renal function: estimated GFR <40

9. Patients treated with hemodialysis
10. Intolerance or contraindications to aspirin or clopidogrel (including thrombocytopenia, prolonged INR)
11. Mini Mental Status Exam score < 24 (adjusted for age and education)
12. Medical contraindication to MRI
13. Pregnancy or women of child-bearing age who are not following an effective method of contraception
14. Pre-existing neurologic, psychiatric, or advanced systemic disease that would confound the neurological or functional outcome evaluations
15. SBP <90 or > 200
16. Known history of limb vascular disease, limb vascular bypass surgery, or limb deep venous thrombosis
17. All women status post mastectomy (standard-of-care re: blood pressure proscription ipsilateral to prior mastectomy)
18. Patients with a contraindication to taking blood pressure in the arm for any reason;
19. Patients with a known bleeding disorder or known abnormality of blood flow to the limb.
20. Patients with osteoporosis or other bone disorders
21. Patients with peripheral nerve injury
22. Patients with abnormal nerve supply
23. Patients with peripheral neuropathy
24. Patients with pre-existing traumatic injury to the limb.
25. Prisoners
26. Homeless individuals
27. Patient unable to give informed consent and no available legally authorized representative to provide informed consent
28. Patient unlikely to be compliant with therapy/ unwilling to return for follow up visits
29. Concurrent participation in another study with investigational drug or device treatment
30. Other likely specific cause of stroke (e.g. dissection, vasculitis, prothrombotic diathesis, drug abuse)

C.4. Enrollment and Consent

After initial patient contact and assessment, the physician-investigator will review the patient's relevant medical history and will verify the diagnosis of small subcortical stroke and moderate to severe WMH and determine eligibility for study entry according to inclusion and exclusion criteria above. Study participants who are competent or who have a legally authorized representative will be enrolled.

C.6. Study Treatment

Randomized Phase: Following signing of the consent form, the patient will be randomized and depending on randomization receive treatment with RPreC once daily, twice daily, or none for 1 year. The RPreC procedure will consist of up to four 5-minute cycles of bilateral upper extremity ischemia, separated by 5-minute periods of reperfusion. Primary outcome measures will be obtained at 1 year.

Follow-up Phase: Patients assigned to RPreC in the first year will exit the trial at the end of Year 1. In contrast, patients assigned to the control arm in the first year will at the start of Year 2 enter a staggered therapy start phase, receiving active RPreC for 1 year (randomized 10 to once daily

and 10 to twice daily), and have additional outcome assessments. The additional data gathered in the Year 2 patients will provide more information regarding intervention feasibility and safety, and also promote adherence to the study protocol in Year 1.

C.7. Concomitant Therapy

All patients enrolled in this trial will receive intensive standard medical secondary prevention stroke treatment as per the recommendations of the AHA/ASA national Guidelines[3]. Patients may not be enrolled in another therapeutic clinical trial until after exit from REM-PROTECT after the two year visit. Patients may not be treated with other experimental stroke therapies

C.8. Device Screening Phase

After a subject consents to participate in the study, he/she will first participate in a study screening phase to ensure a basic level of tolerability of Remote Ischemic Conditioning (autoRIC™) device[4]. The subject will undergo one full cycle of treatment under observation of the study team, including 4 cycles of alternating 5 minute inflation and 5 minute off periods. If the subject indicates willingness to continue receiving such treatment (screening success), she/she will enter the randomized trial phase, and be randomly allocated to the treatment or control group. If subject indicates unwillingness to continue receiving such treatment (screening failure), he/she will not advance to the randomized phase of the trial. Screen failure subjects will be followed up with a 3 day post-device screening phone call to ensure safety and obtain information regarding any adverse events.

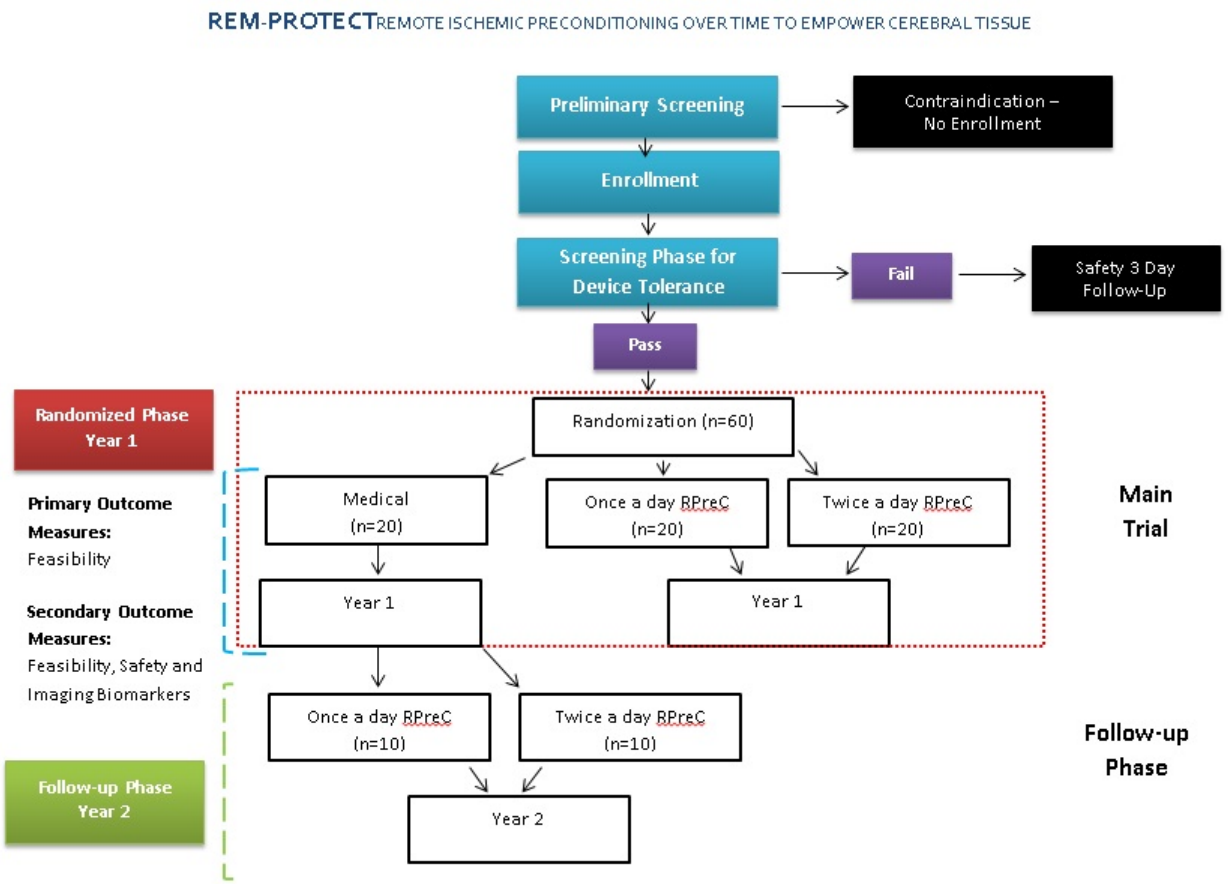


Figure 5. Flow Diagram of the Study Design.

C.9 Study visits

Following the randomization assignment, all patients will have follow-up visits at 1, 3, 6, 9, and 12 months. Feasibility, safety, and efficacy assessments will be performed at each of these visits, with the 12 month measures, at the conclusion of the randomized phase, constituting the primary outcome timepoint. In addition, the 20 control patients will have follow-up visits at 18 and 24 months, during the period of their delayed start therapy. The cognitive assessment battery will be performed at the first qualifying visit to familiarize participants with the system before the baseline assessment at randomization and additionally at 1 month to assess whether any cognitive changes accompanied the initial RPreC effect. If participants ceased study treatment during the study, they will be encouraged to continue attending follow-up visits; all withdrawn participants will be invited to attend the closeout visit to reduce loss to follow-up.

C.10. Treatment Evaluation

C.10.1. Baseline Evaluation

At study entry, enrolling investigator to record:

- Demographic data (age, sex, vascular risk factors)
- Heart rate
- Blood pressure
- Visual inspection of skin in arms, motor and sensory examinations of the hand
- NIH Stroke Scale (NIHSS)
- Modified Rankin Scale (mRS)
- Barthel Index (BI)
- EQ-5D (health-related quality of life)
- Questionaire to verify stroke free status
- Neurocognitive battery
- MR imaging will be reviewed
- Eligibility for enrollment
- Review informed consent with patient

C. 10.2. Day 30 Evaluation

- Interval Events Form
- Concomitant therapies form
- Heart rate
- Blood pressure
- Visual inspection of skin in arms, motor and sensory examinations of the hand
- NIH Stroke Scale (NIHSS)
- Modified Rankin Scale (mRS)
- Barthel Index (BI)
- EQ-5D (health-related quality of life)
- Questionaire to verify stroke free status

- Patient-reported Comfort Rating Scale — a 5 point Likert scale reflecting patient report of subjective experience of RPreC (very comfortable, comfortable, average, uncomfortable, very uncomfortable) (Wang X, Maurin M. Discomfort/Comfort assessment using an ordered category scale.)
- Number of completed 5-minute cycles of RIPC
- Neurocognitive battery

C. 10.3. Month 3, 6, and 9 Evaluations

- Interval Events Form
- Concomitant therapies form
- Heart rate
- Blood pressure
- Visual inspection of skin in arms, motor and sensory examinations of the hand
- NIH Stroke Scale (NIHSS)
- Modified Rankin Scale (mRS)
- Barthel Index (BI)
- EQ-5D (health-related quality of life)
- Questionaire to verify stroke free status
- Patient-reported Comfort Rating Scale — a 5 point Likert scale reflecting patient report of subjective experience of RPreC (very comfortable, comfortable, average, uncomfortable, very uncomfortable) (Wang X, Maurin M. Discomfort/Comfort assessment using an ordered category scale.)
- Number of completed 5-minute cycles of RIPC
- Neurocognitive battery
- Accelerometer assessment of ecologic gait and activity

C. 10.4. Year 1 Evaluation

- Interval Events Form
- Concomitant therapies form
- Heart rate
- Blood pressure
- Visual inspection of skin in arms, motor and sensory examinations of the hand
- NIH Stroke Scale (NIHSS)
- Modified Rankin Scale (mRS)
- Barthel Index (BI)
- EQ-5D (health-related quality of life)
- Questionaire to verify stroke free status
- Patient-reported Comfort Rating Scale — a 5 point Likert scale reflecting patient report of subjective experience of RPreC (very comfortable, comfortable, average, uncomfortable, very uncomfortable) (Wang X, Maurin M. Discomfort/Comfort assessment using an ordered category scale.)
- Number of completed 5-minute cycles of RIPC
- Neurocognitive battery
- Accelerometer assessment of ecologic gait and activity
- MR imaging

In the control group, additional evaluations will be performed at 18 months (same as 6 month visit) and 24 months (same as 12 month visit).

Neurocognitive Battery: The tests performed in the neurocognitive battery are: Montreal Cognitive Assessment (MOCA), Trail Making Test (TMT), Phonemic Fluency (Controlled Oral Word Association Test), Semantic Fluency (Animal Naming), Digit Symbol Coding from the Wechsler Adult Intelligence Scale, Third Edition, Hopkins Verbal Learning Test, Center for the Epidemiological Studies-Depression Scale, Neuropsychiatric Inventory, Questionnaire Version (NPI-Q), Mini Mental Status Exam. These tests are well standardized in the literature and can be administered by study coordinators after training from a clinical neuropsychologist. The test battery has been selected for tests sensitive to attentional, executive, and memory impairments particularly affected by subcortical vascular disease [5].

MR imaging: Sequences performed will include FLAIR, volumetric T1, GRE, diffusion tensor imaging (DTI) and arterial spin labeled (ASL). The total table acquisition time will be 18 minutes.

Measurement of Volumetric MRI Assessment:

All participants will undergo a 1.5-Tesla MRI scanning on the same Magnetom scanner. (Siemens, Erlangen, Germany). The protocol will include the following whole brain scans: 3D T1 MPRAGE imaging (TR/TE/TI 2250/3.68/850 ms; flip angle15º; voxel size 1.0 × 1.0 × 1.0 mm); FLAIR pulse sequences (TR/TE/TI 9000/84/2200 ms; voxel size 1.0 × 1.2 × 5.0 mm, interslice gap 1 mm); DTI (TR/TE 10100/93 ms; voxel size 2.5 × 2.5 × 2.5 mm; 4 unweighted scans, 30 diffusion weighted scans with b-value 900 s/mm²). The complete protocol is estimated to take 35 min.

White matter signal hyperintensities in both supra and infratentorial regions on FLAIR scans, which were not, or only faintly, hypo-intense on T1 weighted images, will be considered WML, except for gliosis surrounding infarcts. WML will be manually segmented on FLAIR images by two trained raters. Total WML volume will be calculated by summing the segmented areas multiplied by slice thickness. Lacunar infarcts were defined as hypo-intense areas with a diameter > 2 mm and < 15 mm with low signal intensity on T1 and FLAIR, ruling out enlarged perivascular spaces and infraputaminal pseudolacunes [6]. All imaging analyses will be performed by raters blinded to clinical information.

Normalization parameters to the ICBM152 linear template (as provided with SPM5; Wellcome Department of Cognitive Neurology, University College London, UK) and gray (GM) and white matter (WM) tissue and cerebrospinal fluid (CSF) probability maps will be computed by using SPM5 unified segmentation routines on the T1 MPRAGE images [7][. Total GM, WM and CSF volumes will be calculated by summing all voxel volumes that had a $p > 0.5$ for belonging to the tissue class. Total brain volume (TBV) will be taken as the sum of total GM and WM. Intracranial volume (ICV) is a summation of all tissue classes, i.e. total GM, total WM and CSF volume. To normalize for head size, TBV will be expressed as percentage of total ICV.

Co-registration parameters of the FLAIR image to the T1 image will be computed (SPM5 mutual information co-registration) and used to bring both the FLAIR and WML segmentation images into the subject's (anatomical) reference frame. Transformed images will be visually checked for

co-registration errors. Subsequently, the WML segmentations will be resampled to and combined with the white matter maps to yield to a WML map (the intersection of WML and white matter) and NAWM map (the complement of WML in white matter) in the T1 reference space.

Diffusion Tensor Imaging Volumetric Analysis

The diffusion weighted images of each subject will be realigned on the unweighted image using mutual information based co registration routines from SPM5. Then, the diffusion tensor and its eigenvalues were computed using an SPM5 add-on (http://sourceforge.net/projects/spmtools)[8]. Unphysical spurious negative eigenvalues of the diffusion tensor will be set to zero, after which the tensor derivatives the MD and FA will be calculated _ENREF_89[9] . The mean unweighted image will be used to compute the co-registration parameters to the anatomical reference T1 image (SPM5 mutual information co-registration), which will then be applied to all diffusion weighted images and derivates. All images will be visually checked for motion artifacts and co registration errors. The mean MD and FA will be calculated in both the WML and NAWM.

Measurement of Cerebral Atrophy

Generalized brain atrophy will be scored on T1-weighted images. Cortical atrophy will be rated on a semiquantitative scale (range 0–15) using reference scans. Subcortical atrophy is measured as the ventricle : brain ratio (range 0.21–0.45) [10]. Estimates of total brain atrophy will be computed primarily from T1-weighted images, bicaudate ratios will be derived from axially acquired images as an estimate of total brain atrophy following established protocols. To derive the bicaudate ratio, the axial slice on which the caudate nuclei produced the greatest amount of indentation on the lateral ventricles will be identified and the distance between the 2 caudate apices will be measured in millimeters. This value is divided by the maximum width of the skull at the same level as the caudate measurement. Using this approach, enlarged ventricles increase the distance between the 2 caudate nuclei, resulting in a higher bicaudate ratio. Therefore, a larger value indicates a greater degree of atrophy. Bicaudate ratio measurements will be made by 2 experienced raters. Interrater reliability will also be computed [11].

C.11. Measures to Avoid and Minimize Bias

Study design considerations

Including a sham device in this study is not a practicable option. Sham inflation to very low levels of pressure would be easily perceived as sham rather than genuine treatment by these older subjects who have substantial experience with blood pressure measurement. Accordingly, low pressure inflations would not protect the blind. Sham inflation to moderate levels of pressure would induce partial limb ischemia and potentially contaminate the control group with a partially active intervention. In addition, the outcome measures in this trial are robust against bias. The primary trial endpoint, tolerability and adherence to treatment in the intervention arms, does not draw upon the control group at all. The MR imaging measures of white matter injury progression, ascertained by automated analysis and blinded readers, are not subject to bias. To protect against bias in ascertainment of the recurrent stroke secondary endpoint, study design includes a) adjudication of potential endpoint stroke events by 2 independent blinded neurologists (preventing rater bias), and b) administration of the Questionnaire to Verify Stroke Free Status at each follow-up visit, to trigger endpoint evaluations (preventing ascertainment bias).

C.12. Blinding

C.12.1 Clinical

The neurocognitive battery and other clinical assessments, including imaging interpretation will be performed by individuals blinded to the treatment allocation of all the subjects to reduce investigator bias. All neurological examinations will be performed by a board-certified neurologist. NIHSS and mRs must be performed by certified study personnel

C.12.2. Imaging

The MR imaging measures of white matter injury progression will be ascertained by automated analysis and blinded readers.

C.13. Adverse Events

Serious adverse events (SAEs) will be identified at every scheduled follow-up point by study site investigators and study nurses. A serious adverse event is one that is fatal or life-threatening, is permanently or substantially disabling, requires or prolongs hospitalization, or is a congenital anomaly (Code of Federal Regulations, Title 21, Chapter ID, Part 312.32), SAEs will be reported to the IRB according to SAE reporting policy. All SAEs should be reported to the REM PROTECT Principal Investigator and the device manufacturer, CellAegis Devices, by telephone, fax, or email within 24 hours of detection.

C. 14. Safety Monitoring

C.14.1. Data and Safety Monitoring Board

To ensure that appropriate ethical consideration is given to the welfare of the patients enrolled in the study, a Data and Safety Monitoring Board (DSMB) will oversee the trial. The DSMB will review the incidences and circumstances of adverse events that occur during the course of the trial. Formal interim analysis will occur at the half way -point, after enrolled patients have completed 50% of patient months of follow up. If the incidence of recurrent stroke or all serious adverse events is higher in the combined device arm than the control arm the study will be placed on hold. The DSMB will conduct a detailed analysis of the safety data. The DSMB may recommend making changes it deems necessary in the interests of the safety or well-being of the study subjects, or suspending or stopping the study. Final determination of a formal stopping rule will be the prerogative of the appointed DSMB.

The members of the DSMB are:

Nerses Sanossian, MD, Stroke Neurologist, University of Southern California
Amytis Towfighi, MD, Stroke Neurologist, Rancho Los Amigos National Rehabilitation Center

C. 14.2. Statistical Design and Analysis Plan

The demographic and baseline clinical characteristics of the study population will be delineated with standard descriptive statistics. Categorical variables describing the clinical history, examination findings, and initial treatment will be summarized by frequencies. Continuous variables such as vital signs, laboratory results, and time variables that follow normal distribution

will be characterized by means, standard deviations, and 95% confidence intervals (CI). Ordinal and non-normally distributed variables (such as the NIHSS) will be characterized by medians and interquartile ranges. RIPC and control patients will be compared to delineate covariate balance. Wilcoxon Rank-Sum tests will be used for continuous or ordinal variables; Fisher's exact tests and chi-square tests will be used for grouped or nominal categorical variables. Comparisons of changes across time (i.e. base to one year) within and between groups will be made using repeated measure analysis variance methods or the non-parametric analog.

C. 14.3. Selection and Analysis of Endpoints

Feasibility: Because the primary aim of this trial is assessing feasibility and tolerability, the primary endpoints are descriptive statistics describing the implementation of the RPreC procedure, including behavioral adherence to treatment, physiologic attainment of limb ischemia, and patient self-reported comfort-discomfort during treatment. The simplicity of these endpoints reduces the likelihood of problems in the analysis and interpretation of these endpoints. The procedure will be considered adequately feasible if adherence and physiologic targets are attained in 75% or more of cases. At this early stage of development, observations and lessons from individual cases may warrant procedural protocol changes to optimize the intervention. Such flexible, developmental iterations of the intervention will be closely discussed with, and supervised by, the study DSMB.

Safety: The lead safety endpoint will be all serious adverse events. Secondary safety endpoints will be deep and superficial vein thrombosis and all cause mortality.

Efficacy: The lead efficacy endpoint will be volumetric progression of white matter ischemic injury on magnetic resonance imaging (biomarker efficacy). The primary MRI endpoint will be the proportion of patients with increase in total white matter hyperintensity volume (as percent of total brain volume) above 1.0%. (This value represents the threshold for the upper quintile of progression in the SCOPE trial. Proportion of patients with progression in the upper quintile was the most sensitive MR endpoint analyzed in the SCOPE substudy.) Additional MRI endpoints analyzed will include mean progression total white matter hyperintensity, mean and quintile progression in deep white matter hyperintensity, progression in total brain atrophy, and progression in DTI anisotropy. The MR imaging measures of white matter injury progression, ascertained by automated analysis and blinded readers, are not subject to bias.

Additional efficacy endpoints explored will include accelerometer assessment of gait quantity and quality in real-world activity; z scores on individual tests and summed performance on the cognitive battery, and incident ischemic stroke, transient ischemic attack, hemorrhagic stroke, myocardial infarction, and cardiovascular death. To protect against bias in ascertainment of the recurrent stroke secondary endpoint, analysis to include a) adjudication of potential endpoint stroke events by 2 independent blinded neurologists (preventing rater bias), and b) administration of the Questionnaire to Verify Stroke Free Status at each follow-up visit, to trigger endpoint evaluations (preventing ascertainment bias).

C.14.4. Sample Size Calculations

Subject size is typical sample size for a feasibility study with a device. We are looking at adherence in order to plan a larger phase II/III successor trial.

Sample Size Justification

The primary feasibility and study endpoint is RPreC adherence at 12 months. As we do not expect clinically important differences in adherence among the three groups, it may be possible to combine two or all three groups. In this study, we also wish to test the null hypothesis that the true adherence is at least 75%. The table below gives the lower 90% or 95% confidence bound for 90%, 95% or 100% observed adherence as a function of sample size and the corresponding statistical power for testing the null hypothesis that the true population adherence is 75% versus the one sided alternative of greater than 75%. The current study is not necessarily sufficiently powered for any of the safety or efficacy outcomes. Therefore this is a pilot study in terms of safety and efficacy.

Lower 90% or 95% one sided confidence bound for true adherence

	adherence=90%		adherence=95%		adherence=100%	
N	90% confidence	95% confidence	90% confidence	95% confidence	90% confidence	95% confidence
20	75.5%	71.8%	81.9%	78.5%	89.1%	86.1%
40	81.0%	78.5%	87.2%	85.1%	94.4%	92.8%
60	83.1%	81.2%	89.2%	87.6%	96.2%	95.1%

Power for one sided test against null hypothesis of 75% vs > 75%

	adherence=90%		adherence=95%		adherence=100%	
N	alpha=0.10	alpha=0.05	alpha=0.10	alpha=0.05	alpha=0.10	alpha=0.05
20	0.677	0.392	0.925	0.736	>0.99	>0.99
40	0.901	0.794	0.997	0.986	>0.99	>0.99
60	0.966	0.927	1.000	0.999	>0.99	>0.99

C.15. Risk Assessment

C.15.1. Formal risk analysis with regard to anticipated risk associated with the device is provided below as per IDE 130167. Risks have been minimized or eliminated through appropriate design control and preclinical testing and bench testing of the device.
The risks associated with the autoRIC are consistent with those of a standard automatic blood pressure cuff, including local bruising, abrasion, and pain at the site of the cuff. Although extremely unlikely to occur, in the case of a serious device failure, hypothetical harms to the patient could include nerve or bone damage.

As presented in Section 1.5 (IDE 130167), previous investigations have reported minimal to no safety implications associated with RIC. In clinical studies where the benefit of RIC was not statistically significant there was no compromise to patient safety. Although the RIC protocol was performed manually in the investigations reported in the literature review, the autoRIC was verified to meet the applicable requirements of standard automated blood pressure cuffs and the performance validation testing conducted on the autoRIC ensures that it performs as intended. As

such, the same performance results can be expected when the RIC protocol is automated by the autoRIC.

C.15.2. Risks associated with MRI:

The MRI scanner does not involve harmful ionizing radiation and is generally well-tolerated. There are no known harmful side-effects associated with temporary exposure to the strong magnetic field used by MRI scanners. There are no known long term risks or complications of MRI. However, there are important safety concerns to consider before performing or undergoing an MRI scan:

1. The magnet may cause pacemakers, artificial limbs, and other implanted medical devices that contain metal to malfunction or heat up during the exam.
2. Any loose metal object may cause damage or injury if it gets pulled toward the magnet.
3. Dyes from tattoos or tattooed eyeliner can cause skin or eye irritation.
4. Medication patches can cause a skin burn.
5. The wire leads used to monitor an electrocardiogram (ECG) trace or respiration during a scan must be placed carefully to avoid causing a skin burn.
6. Prolonged exposure to radio waves during the scan could lead to slight warming of the body.

C.15.3. Other anticipated risks:

Possible complications may occur during the procedure. The complications include but are not limited to, deep venous thrombosis or lymphatic occlusion, petechiae development, skin rash or sensitivity to the cuff, claustrophobia or nerve, bone, or muscle injury. As such, the risk mitigation policy will include, but is not limited to, periodic visual inspection of skin in arms, motor and sensory examinations of the hand to be performed at all office visits.