Study Protocol

Title: Health Evaluation in African Americans using RAS Therapy: The HEART Project

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The HEART Project

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Study Objectives:

Research indicates that certain antihypertensive medications alter Alzheimer's disease (AD) biomarkers in Caucasians. The renin angiotensin system (RAS) regulates blood pressure (BP) in the body and the brain and may directly influence AD biomarkers, including Aβ neuropathology, cerebral blood flow (CBF) and inflammatory markers. This hypothesis is supported by studies, including ours, showing that antihypertensives targeting the RAS reduce the risk and slow progression of AD in Caucasians. We have shown that 1) blood brain barrier (BBB) crossing RAS medications exert protective cognitive and cerebrovascular effects in hypertensive individuals with executive dysfunction and 2) BBB crossing RAS medications are able to significantly alter brain RAS levels in Caucasians 'at risk' for AD due to a parental history.

While mounting evidence supports a protective role of RAS medications in Caucasians, this mechanism has not been explored in African Americans. The rate of AD in African Americans is twice that of Caucasians. Moreover, African Americans are at higher risk hypertension, which likely independently contributes to AD, possibly via RAS regulation. African Americans have low circulating renin and increased aldosterone and sodium levels and are typically not prescribed RAS antihypertensives, *which are those medications believed to exert AD-related benefits in Caucasian*s. If RAS medications are prescribed, high doses are required to achieve BP control, *though the dose needed to alter RAS levels in the brain is unknown*. Because we know that the brain RAS is implicated in AD neuropathology in Caucasians, research should investigate whether RAS medications same also confer AD-related benefits in African Americans. As a first step, we analyzed data from the NIH National Alzheimer's Coordinating Center database in 784 participants with Mild Cognitive Impairment at baseline. We found that African Americans taking RAS acting medications are less likely to convert to AD and exhibit slower cognitive decline than African Americans taking non RAS acting antihypertensives over 3 years. Because these data were retrospective, we were unable to determine whether the brain RAS was the mechanism responsible for these AD related benefits, and thus is the focus of our proposed trial. Importantly, because both Aβ accumulation and RAS dysfunction begin during midlife, it is imperative to investigate these issues in middle-aged, presymptomatic adults, while there is time to stage an intervention, before the irreversible AD cascade begins. Further, exploring the potential benefit of existing drugs that are generally recognized as safe (i.e. "repurposing") such as RAS medications, shortens the time to provide urgently needed treatment options for this devastating illness.

To assess the mechanism by which RAS medications modify the brain RAS, CSF Aβ, cerebral blood flow (CBF) and inflammatory markers in hypertensive African Americans, we propose a Phase Ib double blind, randomized, placebo controlled trial, enrolling 66 middle-aged (30yrs and over), non-demented individuals, at risk for AD by virtue of a family history. Participants will include untreated and treated hypertensives that have never been exposed to a RAS medication. Participants will be randomized by gender and antihypertensive use (y/n) to one of three groups: placebo, or 20mg, or 40mg telmisartan, to determine the dose required to penetrate the CNS.

Our overarching hypothesis is that, compared to placebo, both doses of telmisartan will penetrate the CNS and produce salutary, *dose dependent* effects on the brain RAS as well as CSF Aβ, CBF and CSF inflammatory markers in African Americans, over eight months.

Aim 1: Establish the dose of telmisartan necessary to penetrate the CNS and to significantly alter components of the brain RAS in 66 African Americans with a family history of AD.

HYPOTHESIS: We hypothesize that, compared to placebo, both doses of telmisartan will penetrate the CNS and that we will observe a dose effect of telmisartan's ability to significantly alter brain RAS function.

Aim 2: Determine whether telmisartan significantly alters CSF Aβ, CBF and CSF inflammatory markers.

HYPOTHESIS: We hypothesize that, compared to placebo, both doses of telmisartan will produce slower decline in CSF Aβ levels, CBF and lower inflammation, and that we will observe a dose dependent effect of 20mg vs 40mg telmisartan, over eight months.

2. BACKGROUND AND SIGNIFICANCE

2. 1. Physiological Changes in Preclinical AD and the Importance of Prevention: Use of certain antihypertensive medications during midlife is associated with cognitive decline and Alzheimer's disease (AD). Specifically, blood Pressure (BP) medications acting via the renin angiotensin system (RAS) have been implicated in Aβ neuropathology, cerebrovascular regulation, and inflammation – all of which have been identified as early change mechanisms in AD. Therapies that *preserve or improve brain RAS function may reduce early AD related neuropathology* may interrupt this cascade to delay the development of AD pathology.

2. 2. Brain RAS dysfunction increases the risk of Alzheimer's disease: Angiotensin converting enzyme (ACE) is a central component of the RAS system and has been directly implicated an Aβ neuropathology in Caucasians. The role of ACE is to convert angiotensin I (Ang I) to angiotensin II (Ang II), which is a potent vasoconstrictor. Caucasians with MCI and early AD exhibit elevated ACE activity in cerebrospinal fluid (CSF) and hypoperfusion in the parietal cortex, a region expressing Ang I and Ang II receptors and therefore a potential site for increased vasoconstriction in early AD. Studies involving postmortem tissue suggest that the RAS itself seems to be significantly altered in AD, such that the tissue exhibits elevated ACE activity, which correlates with AD severity and parenchymal Aβ load **(See Preliminary Data)**[1]. Moreover, increased ACE activity reportedly mediates decreases in acetylcholine release via increased Ang II production, which may also contribute to AD-related cognitive decline. Thus, medications designed to target ACE activity in the brain, and subsequent reduction of Ang II, could potentially serve as an alternative AD treatment via direct actions on Aβ pathology CBF and inflammation. This hypothesis is supported by studies, including ours **(Preliminary Data),** reporting that treatment with centrally acting RAS medications (ACE inhibitors (ACE-I) and angiotensin II receptor blockers (ARBs)) have the ability to reduce ACE activity in CSF and may reduce the likelihood or slow the progression of AD in Caucasians.

2. 3. Racial Discrepancies in RAS function: Research documenting blood pressure (BP) and systemic RAS differences between African Americans and Caucasians has existed for decades. African Americans have low circulating renin levels and increased aldosterone and sodium levels, which has led some to believe that, in African Americans, the RAS is not the primary system involved in systemic vascular regulation. While the importance of systemic RAS function in African Americans is still under investigation, no studies have been conducted to investigate the potential importance of the brain RAS in African Americans. Because we know that the brain RAS plays an important role in AD pathology and cognition in Caucasians, this mechanism needs to be explored in African Americans, who stand to benefit the most from AD therapies targeting the brain RAS.

2. 4. Blood Pressure Medication Prescription Practices: Discrepancies exist in the types of BP medications prescribed to Caucasians vs. African Americans based in part on past studies on past studies of clinical efficacy and the overall belief that the RAS may be of little importance in African Americans. If certain BP medications are found to beneficially influence AD biomarkers and hence AD risk – it is important to understand this mechanistic relationship in the presence of potential racial differences in RAS function and differences in BP medication assignment between African Americans and Caucasians. Research shows that, compared to Caucasians, African Americans are more likely to be prescribed antihypertensive therapy in general, and more likely to be prescribed aggressive therapies and combination regimens. Also, African Americans are more often prescribed calcium channel blockers and diuretics, while Caucasians are often prescribed RAS acting antihypertensives – those medications implicated in having salutary effects on cognition and AD incidence, independent of their effect on BP reduction. While many researchers now argue that the racial prescription practices are unwarranted, these trends persist in clinical practice and were recently upheld in the Guidelines for the treatment of hypertension in February, 2014, and thus is a central component the proposed project.

2. 5 Significance: While evidence linking the RAS and AD continues to mount, there are no therapeutic strategies targeting the RAS to prevent or delay AD. This Phase Ib RCT is the first step in understanding the potential of RAS acting medications in regard to CNS penetration and AD biomarkers with the future goal of repurposing an existing, inexpensive, safe drug. African Americans are not generally prescribed RAS medications, but when they are, doses higher than those prescribed to Caucasians are required to achieve BP control. It is unknown how these high doses alter systemic RAS levels or what dose is adequate to penetrate the CNS and alter brain RAS levels in African Americans. *The dose is likely to be LOWER than the dose needed to alter BP, which is the case in Caucasians.* Targeting individuals at high risk will help prevent and slow AD progression in as many people as possible, as fast as possible. Toward this goal, we must first understand the mechanistic potential of these medications in regard to the dose required to achieve CNS permeability, while safely controlling BP levels.

3. INNOVATION

3. 1. This study will provide POC and data for power calculations for Phase IV clinical trials toward the goal of repurposing safe, inexpensive, RAS targeting medications for use in AD prevention trials in high risk populations. This trial will investigate the potential of an existing, affordable FDA approved medication as a potential therapy for AD prevention. Because research shows that higher doses of RAS acting medications are needed to achieve adequate BP control, we are testing whether telmisartan can penetrate the CNS and alter brain RAS indices irrespective of changes in BP. In doing this –telmisartan could be tested in larger phase IV clinical trials to determine whether similar pharmacological therapies may be helpful in preventing AD in African Americans, and whether these therapies could be used in addition to existing BP regimens.

3. 2. This study will explore the role of brain RAS function in African Americans: While African Americans represent only 13% of the U.S. population; they comprise 1/3 of the cost incurred from AD, half of which is concentrated in the South. African Americans comprise 61.4% of the population of Atlanta and 28.7% of the Georgia population. Thus, this trial has a unique opportunity to examine the influence of RAS medications in this high risk group. Emory is located in the “Stroke Belt” which has a high incidence of morbidity and mortality from cerebrovascular disease. In addition to having a two-fold risk for AD, African Americans are at increased risk for a host of vascular disorders. By characterizing the brain RAS, and its response to RAS medications in African Americans, we stand to glean invaluable information that could be used for prevention and treatment of vascular disease as well as AD and vascular dementia.

3. 3. We will implement a state-of-the-art methodology to ascertain all components of the brain RAS. The RAS-fingerprint™ is a multiplex parameter designed and developed for the comprehensive analysis of the renin angiotensin system. Ten different angiotensin metabolites are measured simultaneously in a single sample of less than 1ml CSF. This methodology will provide optimal characterization of the RAS before and after drug, which, when coupled with the RAS measures we are collecting in plasma – will provide the most comprehensive characterization of systemic and brain RAS function in African Americans to date.

4. APPROACH

4. 1. Preliminary Data: Research [2-6] conducted by the PI and Key Personnel has led to the development of the project. Studies demonstrate productivity, feasibility and provide preliminary results suggesting that regulation of the brain RAS is a risk factor for cognitive impairment and longitudinal decline and may have AD-modifying potential in African Americans. This Phase 1b trial will clarify these research questions in African Americans with a family history of AD.

4. 2. Studies supporting a link between RAS function and AD pathobiology: Dr. Whitney Wharton **(PI)** has reported that centrally acting ACE-Is have the ability to alter CSF ACE activity and these changes are detectable in middle aged Caucasians at risk for AD [6]. Dr. Patrick Kehoe **(Key Person)** has reported that

therapies targeting the RAS likely improve diminished CBF, which is common in AD and can contribute to Aβ pathology. Importantly, while some studies support a beneficial effect of ACE-I on Aβ, others report that ACE may have the potential to degrade Aβ, though the cleavage site is still under investigation and this has not been confirmed in humans. This issue is exceedingly important and this project is designed to address this question in at-risk individuals.

4. 3. Studies supporting less amyloid deposition in Caucasians treated with RAS acting antihypertensives: Dr. Ihab Hajjar (**Key Person)** analyzed data from the NACC cohort (N = 890) and has reported that individuals with AD treated with RAS acting therapy showed less amyloid deposition markers upon autopsy compared to participants treated with other antihypertensive medications [7].

4. 4. Studies supporting less disease conversion and slower cognitive decline in MCI participants treated with RAS medications. We recently analyzed data from the National Alzheimer's Coordinating Committee (NACC) database in 8187 participants who had at least 3 annual cognitive testing sessions (publication in revision). Participants taking RAS acting medications (N = 488) were less likely to convert to AD ($p = 0.04$) and had slower decline on the CDR Sum of Boxes (SOB) ($p < 0.01$) and digit span forward ($p = 0.02$) than participants taking non-RAS acting antihypertensives. Moreover, BBB crossing RAS antihypertensives users (N=312) exhibited slower cognitive decline on multiple tests of cognitive and functional ability compared to non BBB crossing RAS users. RAS acting medications were associated with beneficial cognitive effects in both African Americans and Caucasians. African Americans had more pronounced benefits relative to Caucasians on the MMSE ($p = .05$) category fluency ($p = 0.04$) and Digit Span Backwards ($p = 0.03$) tests. These important findings suggests that the brain RAS plays a role in disease conversion and AD related cognitive function in African Americans, and highlights the need to address the neurobiological underpinnings of this relationship.

4. 5. Summary of Preliminary Data: Our data indicate that African Americans and Caucasians at-risk for AD demonstrate pathological and cognitive changes suggestive of preclinical disease, and that these changes are likely influenced, at least in part, RAS function in the brain during midlife, which is both detectable and *modifiable*. While our results shed some light on *individual* components to be addressed in the proposed trial, the interplay among the factors (brain RAS activity, race, and AD biomarkers) has yet to be explored.

TABLE 1. INCLUSION / EXCLUSION CRITERIA

INCLUSION CRITERIA
Age over 30 years
Mean Resting Blood Pressure ≥110 systolic and ≤ 170 mmHg systolic
Family History of Alzheimer's disease[1] (i.e., biological parent, grandparent, or sibling) [1]exceptions can be made at the discretion of the PI
African American
EXCLUSION CRITERIA
Currently in another investigational drug study
Potassium > 5.0 meg/dL at baseline
Creatinine > 1.99 mg/dL at baseline
History of stroke or TIA
Dementia
Current use of a RAS acting medication
Contraindication for LP or MRI[2] [2]exceptions can be made at the discretion of the PI to enroll participant and exclude LP or MRI procedure if contraindicated or procedure refused
Heart Failure
Diabetes Types I & II
Pregnant or nursing women

5. RESEARCH DESIGN AND METHODS

5.1. Participants: Sixty-six non-Hispanic African American participants from the Emory ADRC will be enrolled in this trial. Inclusion and exclusion criteria are outlined in **Table 1.** We will enroll African Americans age 30 and over, all of whom will have a family history of AD. Participants must not be taking a RAS acting antihypertensive and their BP must be ≥110 and ≤ 170 mmHg systolic at both the screening and Baseline visits. Participants were selected because they are at high risk for AD and would most likely benefit from an intervention. The ADRC cohort contains a unique registry of individuals that have a parent with either autopsy-confirmed or probable AD as defined by NINDS-ADRDA criteria. Familial AD diagnosis will be verified using the validated Dementia Questionnaire and medical records when available. Exceptions to these guidelines will be rare but may be considered on a case-by-case basis at the discretion of the PI in consultation a Study Physician (Dr. William Hu or Dr. Lynn Marie Trotti). Additionally, at PI discretion, participants with a contraindication for LP or MRI may be enrolled with contraindicated or refused procedure omitted in order to expand enrollment pool.

Recruitment

Participants will be recruited directly from the ADRC cohort and via the following:

Community-based recruitment: This will include announcement and recruitment information in periodicals and local newspapers. Blood pressure education sessions in local communities e.g. churches or barbershops will also be conducted.

Physician recruitment: Local physicians (primary care or specialty physicians) will be informed of the study and its requirements and provided information about referral to the study personnel. In addition, flyers for the study will be posted at outpatient areas in the following facilities: Emory University Hospital, Emory Clinic, Grady Memorial Hospital, and the VA medical Center if possible and all will be approached for the project. Of note, the PI has recruited this population in her ongoing past observational and clinical trials and reached her recruitment goal within the award period, supporting the feasibility of our recruitment goal. This enrollment rate allows us ample time to finish recruitment two years into the 5-year grant period and complete preliminary analyses by end of year 2 to support submission of a NIA R01 application in 2018. Also, we will write manuscripts using this data soon after analyses are completed.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Participants will be randomized by gender and antihypertensive use (y/n) into either telmisartan (20mg or 40mg) or placebo groups. Treatment will be provided in a capsule format to be taken once a day orally. All three medication arms will be formulated into identical capsules. Investigators, study personnel and participants will be blinded for drug assignment. Participants will be on medication for 8 months. A study physician Dr. William H, MD, PhD or Dr. Lynn Marie Trotti will ensure participants blood pressure does not fall to unsafe levels and if a participant's blood pressure is too low (systolic <90 mmHg), we will contact the participant and the participant's PCP.

5.2 Handling of Study Interventions

The drugs will be stored in the medication room in the Investigational Drug Service (IDS) Pharmacy. Standardized randomization by gender and active antihypertensive use (y/n) was calculated by John Hanfelt, the HEART Project biostatistician. The location of the IDS pharmacy is The Emory Clinic Bldg. A, Suite 1200, 1365 Clifton Road, NE, Atlanta, Georgia 30322. Access to the med room is limited to the IDS Pharmacists via a badge swipe. Accountability records are maintained for all investigational product (IP). A courier will deliver prescriptions to study location to give to subjects or study staff will be able to pick up prescriptions. Subjects will receive four months of pills for their baseline visit and four months' worth during at their 4 Month follow-up. Study coordinators will hand study medication to participants, but will not answer any questions about study medication. All questions about study medication will be answered by study physician or nurse practitioner during the visit or by using the contact information on the study medication label.

5.3 Concomitant Interventions

The goal of this Phase I trial is to safely assess the BBB permeability of low and clinical starting doses of an FDA approved ARB. Participants who are currently taking a RAS acting medication, (ACE-I or AR2RB) will not be enrolled. The clinical starting dose of Telmisartan for BP control in Caucasians is 40mg. This study will test the BBB permeability at 20mg vs 40mg vs placebo. Participants taking antihypertensives will be instructed to continue their current BP medication regimen. All participants' PCP will be sent a letter describing the HEART Project and will be contacted by a study physician, Dr. William Hu or Dr. Lynn Marie Trotti, if their medication needs to be altered.

5.4 Allowed Interventions

Participants will continue to receive their usual care from their regular primary physicians. Hypertension management will be addressed by the study physician only insofar as communication with the participant's PCP. The purpose of this Phase I RTC is not to control BP, therefore, we will not assume control of the participants BP maintenance as long as they are within safe limits.

5.5 Required Interventions

In addition to the study medication (telmisartan or placebo), other non-RAS acting medications (e.g. HCTZ, amlodipine, and metoprolol) are allowed to achieve blood pressure control to below 140/90 mm Hg. All participants will remain on their medications as prescribed by their PCP in addition to taking the study medication or placebo pill.

5.6 Prohibited Interventions

Once participants are enrolled, addition of RAS acting antihypertensive medications by non-study providers is not allowed. However, non-RAS acting medications and non-hypertensive medications are allowed as part of usual care.

5.7 Adherence Assessment

Participants will be asked to bring their study medication bottles to the study center at each visit. Medication compliance will be assessed using pill count at the safety visits, Month 4 and Month 8. We will define a compliance rate for a time period, t, as the ratio of: (the used number of pill prescribed for the number of days t- number of pills remaining or unused for the time t/ number of pills prescribed for time t) multiplied by 100.

Data to be collected in the proposed study:

Blood Test:

Blood chemistries including a Basic Metabolic Panel (glucose; calcium; electrolytes: sodium, potassium, carbon dioxide, bicarbonate, chloride; kidney tests: BUN, creatinine) and a CBC (Complete Blood Count) will be measured at Screening and optionally at Baseline and Safety Visit(s) per study doctor's discretion as part of the safety monitoring for hyperkalemia and renal insufficiency. We estimate that only a subset of 33 subjects will have the basic metabolic panel and CBC performed on more than one Safety Visit. Blood will be drawn after application of a tourniquet, by use of a small gauge needle, from an ante-cubital vein, and by an appropriately trained professional from subjects.

Additionally, research blood will be drawn at Baseline, Month 4, and Month 8 visits, including for APOE genotyping. Standard PCR and DNA sequencing techniques are used in a CLIA certified lab using the FinchTV program and not divulged to participants. We will utilize existing resources to assess levels of systemic RAS function in blood, including aldosterone, angiotensinogen, renin and ACE, to determine the relationship between circulating and brain RAS function among African Americans.

Arterial Function Measure via Ultrasound – PWV: Pulse wave velocity (PWV) is non-invasive and will be administered at Baseline and Month 8. PWV is a measure of artery wall stiffness, is highly reproducible and has a strong correlation with cardiovascular events and all-cause mortality. Dr. Wharton collects these measures in her ongoing NIH funded study and is familiar with the procedures.

Neuropsychological testing: Testing will ensure a dementia-free diagnosis and will be used to determine the extent to which brain RAS function affects cognition in African Americans using a comprehensive battery in several cognitive domains (memory, executive function and language), with a focus on executive function.

These tests are currently being used in the PIs ongoing NIH funded study and were selected for our cognitively normal, middle aged sample and because data show they are particularly sensitive to fluctuation in blood pressure.

Executive function will be assessed using the NINDS-initiated EXecutive Abilities: Measures and Instruments for Neurobehavioral Evaluation and Research or "EXAMINER" tool box. This test battery reliably and validly assesses executive function in clinical trials [http://examiner.ucsf.edu/].[115-117] The battery includes 11 tasks that generate 15 primary variables. Within this set, the EXAMINER investigators identified tasks measuring four domains that fit well in a single factor model of executive function: working memory, inhibition, set shifting, and fluency. The EXAMINER battery has excellent psychometric properties with test-retest reliability of over 0.9 and correlates by over 0.60 with an informant-based measure of day-to-day executive functioning, the Frontal Systems Behavior Scale. The parts of EXAMINER that we selected include:

1) Flanker task (inhibition) which involves responding to a central stimulus while ignoring flanking stimuli that are either compatible or incompatible with the central stimulus.[118]
2) Set-shifting, a measure of mental flexibility assessing the subject's ability to attend to the specific attributes of compound stimuli, and to shift that attention when required.[119]
3) Spatial N-Back test assesses spatial working memory

Data will be scored using an open source web-based data management and scoring system available through the EXAMINER project Using item response theory, a single composite executive function score will be calculated from the available data points.[117]

To assess additional cognitive domains/mood, we will use these tests included in the National Alzheimer's Coordinating Center Uniform Data Set.[122,123]

Tests include (but are not limited to): MOCA, MMSE, WAIS-Working Memory subtests, WMS-III Faces, Brief Visuospatial Memory Test-Revised, WMS-R Logical Memory, and the Rey Auditory Verbal Learning Test. By utilizing the results of the AT RISK assessment of repeat testing, the project is more cost efficient and ensures that participants are not overburdened and practice effects are avoided. If an effect of uncontrolled BP levels is observed, we predict that they will manifest on executive function tasks. Of all the cognitive domains, executive function is more vulnerable to hypertension and is selectively impaired in early AD associated cognitive decline

Ambulatory Blood Pressure Monitoring: Ambulatory measures provide a superior predictive value for cardiovascular events compared to clinic BP measures and have been used in prior AD research including the PI's projects. These data will add insight into the relationship between BP and brain RAS in African Americans. This procedure is optional due to the difficultly of returning the ABPM equipment for some participants. Procedure will be omitted only for participants who cannot return monitor.

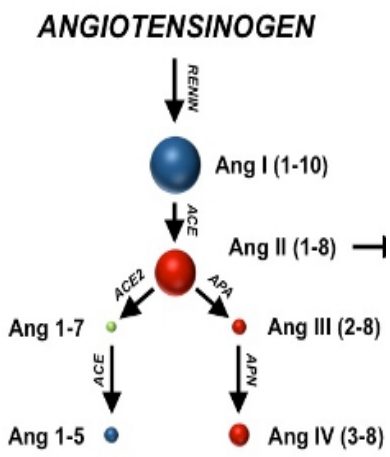
CSF Biomarkers: CSF samples (Aβ and RAS Fingerprinting) will be collected at Baseline and Month 8 by a HEART study team member using a 24 gauge Sprotte needle and a gentle extraction technique widely used in CSF biomarker research in AD. About 22ml of CSF will be collected, aliquoted and stored at -80°C until time of analyses. Aβ42 will be measured using xMAP technology by the ADRC team who are experts in AD biomarkers. Visits will occur in a designated clinical room at the ADRC memory clinic at 12 Executive Park or the ACTSI in the EUH. All CSF samples will be collected after an 8-hour overnight fast. A member of the ADRC biomarker team will perform all LPs.

All CSF collection is completed according to guidelines put forth in the "Biospecimens Best Practice Guidelines for the ADCs" published by the National Alzheimer's Coordinating Center (NACC) and available on their website. CSF collection occurs at approximately the same time at each visit to control for diurnal variability. Participants are placed in the sitting position and asked to maximally flex their knees, hips, back, and neck. The skin over L4-L5 is prepped and draped in a sterile manner. 1% lidocaine is used as a local anesthetic, followed by insertion of a spinal needle with introducer into the L4-L5 interspace using sterile technique.

Approximately 25ml of CSF will be collected using sterile polypropylene collection tubes. The samples will be immediately divided into aliquots and transferred for storage in a freezer for future assays. Risks of LP are discussed under *Protection of Human Subjects*.

RAS analysis in CSF: CSF samples will be collected in all participants. State of the art technology will measure 10 individual components of the brain RAS system including Analyses will be performed by Dr. Marko Poglitsch in Austria, developer of the RAS-Fingerprint™ technology http://www.attoquant.com/sites/info_renin.html **(Figure 2).**

Figure 2



Tau and Abeta: CSF T-tau, and P-tau$_{181,}$ Aβ_{40} and Aβ_{42} and other proteins related to Alzheimer's disease risk will be measured by Dr. Henrik Zetterberg in Sweden using XMap technology. Dr. Zetterberg is a nationally recognized biofluid and Alzheimer's disease expert and Dr. Wharton has collaborated with him and utilized her services and expertise in all of her current and past studies. All samples will be coded with a unique identifier to keep the laboratory blinded to factors such as subject identity or treatment group.

Neuroimaging: The sequence is fasting takes one hour. The primary scan of interest is ASL, and T1, T2, T2 FLAIR, and DTI are also measured. Data is acquired while the participant is awake with no cognitive task or contrast. All scans are FDA approved. Flow images are manually processed with in-house developed Matlab & C programs. The sequence is described as follows:

(i) ASL-MRI: ASL methods were previously described.[137,138] Briefly, echoplanar T1 mapping scans will be used for image registration and a scout image of the head will be obtained in order to choose the appropriate location for spin labeling and flow imaging. ASL images will be acquired using a custom 3D stack of interleaved spirals fast spin echo sequence and will be averaged in order to improve the signal-to-noise ratio. An algorithm to remove subtle motion artifacts will be used for individual images prior to signal averaging.[137-140] Images will be interpolated and smoothed in a panel by using a 0.5-pixel, full-width, half-maximum Gaussian kernel. Perfusion values will be calculated as describe elsewhere[138] and converted to ml/100g/min. Regional perfusion will be quantified in each hemisphere.[141] Maps of cerebral vasoreactivity will be obtained by co-registration of perfusion and anatomical images allowing us to have perfusion and vasoreactivity maps before and after treatment. [137-140] ASL reliability is high with ICC ≥0.75.[142]

(ii) Structural MRI and WMH: High-resolution anatomical images will be acquired using a 3D-Fast Spoiled Gradient Recalled Echo (FSPGR) Sequence. WMH regions will be identified by a 3D T2 Fluid Attenuated Inversion Recovery (FLAIR) Fast Spin Echo sequence. Obtained images will be co-registered using a within-subject inter-modal alignment between structural and perfusion images. T1-weighted SPGR images will be used to identify cerebrospinal fluid and white and gray matter (WM, GM), both global and regional. The FreeSurfer analysis suite (http://surfer.nmr.mgh.harvard.edu/) will be used to conduct automated extraction of volumes of targeted regions (in mm3) including hippocampal volume.[129-131] Hippocampal volume will be used as an index of Alzheimer's disease (AD) pathology.[132] WMH volumes (in mm3) will be obtained from FLAIR imaging according to a semi-automated analysis protocol developed and validated by DeCarli et.al.[133,134] The reliability assessed by the intra class correlation (ICC) is 0.79 to 0.85[135] for regional volumes and 0.99 for WMH.[136] All volumes will be adjusted for intracranial volume in our analyses.

(iii) Diffusion tensor imaging and tractography: DTI is ideal in our study because it is highly sensitive to detect structural changes over a short time, less than 1 year, as compared to traditional MRI.[59] DTI is based on the imaging of water molecules' diffusion and direction. (see review[143]) Microstructural damage in WM will be quantified using voxel based morphometry (VBM) of diffusion tensor metrics including mean diffusivity (MD), which is a measure of the extent of diffusion, the fractional anisotropy (FA), which measures the difference among the tensor eigen values reflecting microstructural integrity, and axial and radial diffusivity, which

measure diffusion or structural integrity along the axial and radial directions respectively. These measures are highly reliable with ICC ≥0.88 for FA, and ICC ≥0.84 for MD values,[144,145] and increased MD and decreased FA are highly correlated with executive function.[146] A novel Independent Component Analysis (ICA) based multi-fiber-per-voxel streamline tractography developed by Dr. Singh[147] will be used to quantify microstructural changes along specific tracts known to be involved in executive function including the fronto-occipital, cingulum and uncinate. Whole-brain multi-fiber-per-voxel tracts in the native space of each subject will be mapped to the T1 template space using the DARTEL transformation, and a statistical analysis of diffusion metrics of voxels lying along specific pathways (sorted in a common template space for all subjects) will be performed. For each participant baseline and post treatment images will be compared qualitatively as well as quantitatively using pairwise voxel-based comparisons (Statistical Parametric Mapping (SPM8), Wellcome Trust Center for Neuroimaging, UK) for the various within tract metrics described by Correia et al.[148]

(iv) Resting functional MRI (r-fMRI): This protocol, developed in the last few years, is designed to assess the communication or "functional connectivity" between brain regions.[149] Functional connectivity has a key role in important complex cognitive processes.[149] It focuses on spontaneous, rather than task-induced, low frequency (<0.1 Hz) fluctuations in the blood oxygenation level-dependent (BOLD) signals. r-fMRI supplements structural connectivity measured by DTI (see above) with functional connectivity of the brain. This method avoids performance related variability seen in activation fMRI, is less complicated to acquire and standardize, does not require radio-isotopes, and may be more effective at identifying functional pathology associated with risk of future cognitive decline compared to non-resting fMRI techniques.[150] Participants are instructed to lay still with eyes closed and remain motionless as much as possible while a series of images is acquired for 6 minutes. The first 10 image acquisitions of the resting state will be excluded from analysis to allow for adaptation of the subjects to the circumstances. The remaining images will be motion-corrected co-registered as mentioned previously. The functional images will be re-sampled to 3-mm isotropic voxels followed by spatial smoothing with a 4-mm full width at half maximum (FWHM) Gaussian kernel. Finally, temporal filtering (0.01 Hz < f < 0.08 Hz) will be performed on the time series of each voxel to reduce the effect of low-frequency drifts and high-frequency noise.[151] Other image processing includes time shifts and motion correction; removal of cardiac, respiratory, white matter, and cerebrospinal fluid signal effect and applying a low-frequency filtering. A seed based functional connectivity analysis (model-dependent) will be performed by calculating the correlation between the BOLD time series activity in one or several a priori voxel region(s)-of-interest (ROI),or “seed region”, and activity in all other time series activity voxels in the brain.[151,152] Voxels with high degree of correlation form a connectivity map or a "resting functional connectivity map". [153] Prior studies suggest that the brain is comprised of few global networks or hubs that are intimately connected.[154] Using this seed-region method we will assess brain connectivity in the dorsal medial prefrontal cortex subsystem which is involved in executive cognitive function[155-158] which is likely to be affected DM and hypertension.[159,160] More recent methods have been used that are independent of an a-priori ROI which overcomes the drawback of missing a potential target region or tract. Using graph theory, metrics can be derived about brain functional connectivity based on the distance between vertices (voxels) and the degree of connectivity (number of edges connecting to the voxel).[161,162] Using this method, we can derive a matrix that reflects overall connectivity and efficiency of brain function: N=number of nodes; k=mean degree; d=connection density; C=clustering coefficient; L=path length; λ=path length scaled to random graph; γ=clustering coefficient scaled to random graph; σ=scalar small-worldness measure.[161,162]

F. 7. Tissue Banking

We will collect blood and CSF for future research. Tissue banking will be accomplished with the assistance of HEART Project, ADRC and staff and facilities. Vials containing blood samples will be labeled with subject ID numbers, name of the study, date collected, barcoded and temporarily stored at the EP6, Rollins, or Whitehead buildings. The label will include the study name, study visit number, contents of each collection vial, and the date of collection. Tubes will be labeled with a barcode by an ADRC Technician. All samples from this protocol will be kept in a research freezer in a secure research freezer space located in the EP6, Rollins or Whitehead building. Access to the freezer is protected, and equipped with backup power and a telephone alarm system. The research freezer space can only be accessed by authorized study personnel.

All samples are collected for research purposes only. If blood is used for future assays, blood remaining after completion of laboratory tests will be discarded. Any genotype information obtained from blood will be stored in a coded and secure database, located in the ADRC and HEART Project lab space. Only authorized and certified study personnel will have access to the code which will be held in a password protected database on the Emory network server, access to which will be restricted to small number of approved study personnel.

Tissue specimen banking (blood and CSF) is not an optional part of the study. The samples will be kept until all analysis for this project is complete or until the samples are exhausted. Future study results obtained from these samples may not be reported to subjects. Subjects (or their designated representative) wishing to withdraw their consent to bank specimens will need to contact the PI with a written request to have stored samples destroyed (autoclaved). If the PI leaves Emory University, the samples will stay with Emory (not leave with the PI).

Having these additional banked samples will allow for future analysis of Alzheimer's disease or other disease biomarkers if/when new analytical technique for analysis for these samples becomes available. Furthermore, it allows for comparison between various techniques for analysis of these samples, thus furthering the research in this area.

F.9 Questionnaires and handouts

F. 9. 1. Personal Health Questionnaire Depression Scale (PHQ-8)): This standardized tool measures depression. If participant scores 10 or greater its considered major depression, 20 or more is severe depression., The PI will be notified at once, who will then notify an ADRC clinical neuropsychologist who will contact the participant.

F. 9. 2. Exercise: This short questionnaire asks about the participant's exercise routine, specifically cardiovascular activities and weight-lifting. The exercise history questionnaires assess normal activity levels, which is important when assessing blood pressure patterns.

F. 9. 3. Medical: This questionnaire asks about alcohol and smoking history, education and other demographics, surgeries, medications, allergies, and current symptoms.

F. 9. 4. Sleep: This questionnaire asks about normal sleep patterns on work and non-work days, about snoring, sleep apnea, and other sleep problems. These problems can affect blood pressure and cognition.

F. 9. 5. Blood Pressure Information Handout: This handout explains basic information about blood pressure, why it is important, and how it can be lowered

F. 9. 6. Diary form: this form allows the participant to keep a diary of their activities while wearing the blood pressure cuff.

F. 9. 7. Nutrition Survey: This survey is an online diet questionnaire and is optional. The decline to complete this survey will not affect the participant study participation.

F. 9. 8. Stress Questionnaires: These questionnaires include but are not limited to mood, stress, the quality of life of your family member with Alzheimer's disease, nutrition, and exercise.

F. 9. 9. Perceived Discrimination Survey Battery: This battery of questionnaires consists of: Chronic Work Discrimination and Harrassment Scale; Coping with Discrimination Scale; Heightened Vigilance Scale: Major Experiences of Discrimination Scale; The Expanded Everyday Discrimination Scale. These surveys will be administered online, are optional, and will not affect study participation if declined.

5. 2 Procedures*:*

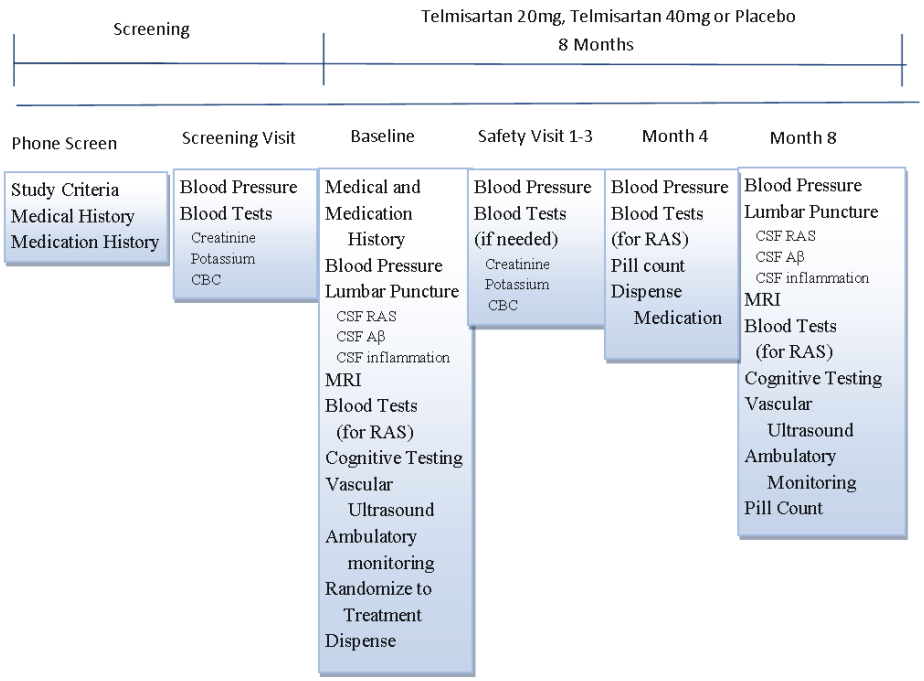
5. 2. 1. Intervention and randomization: We will recruit 66 participants to account for a potential 10% drop out rate over the 8 month trial. Standardized randomization by the study biostatistician will be used to assign participants by gender and antihypertensive use (y/n)by the Emory Pharmaceutical Research Center to receive 20mg telmisartan (N = 22), 40mg telmisartan (N = 22) or placebo (N = 22) to be taken once daily before bed. Study drug and placebo will be formulated into identical capsules. The PI, study personnel and participants will be blinded for drug assignment. A study physician will ensure BP control and will be the only unblinded study

personnel. Participants are not required to stop their current medications to test this POC, mechanistic research question (i.e. pre vs. post RAS medication exposure and CNS permeability). Participants requiring additional BP medication in addition to study drug, or participants whose BP values drop too low will be referred to their PCP in addition to HEART Project personnel contacting the participant's PCP to request medication alteration for the duration of the trial. If a participant requires an additional medication, supplemental antihypertensives will not act via the RAS. Participants will return every 2 weeks until control is established.

5. 2. 2. Study Visit Description Summary: Participants will complete approximately 8 visits over the course of the 8 month trial depending on the number of Safety visits required. (**Figure 1**). We will utilize the Emory ADRC participant registry and contact the adult children who are study partners of individuals who have an AD diagnosis. First, a verbal consent and a phone interview will be conducted which will determine eligibility and introduce the study protocol using standardized questions. AD diagnosis is verified using the diagnosed family member's medical records or the validated Dementia Questionnaire (DQ). Based on the make-up of the identical Wisconsin ADRC AT RISK cohort, we predict that approximately 45% of participants with a family history of AD are APOE4 positive.

Those who are eligible will be invited for a screening visit, which includes: obtaining informed consent, cognitive screening, medical history, current medications, 2 seated BP measurements and baseline metabolic blood panel and CBC. Participants who are untreated hypertensives or currently taking BP medication are eligible *as long as existing therapies do not act via the RAS*. After this screening, eligible participants are asked to return for a baseline evaluation and randomization. Baseline and month 8 visits are fasting for 8 hours prior to the visit, take approximately 4 hours and consists of BP measurements, physical exam, blood draw, lumbar puncture, brain MRI, vascular ultrasound, ambulatory BP monitoring (optional) and cognitive testing. Randomization and treatment will start after baseline data collection.

Figure 1



Participants will then be seen in approximately two weeks following baseline for their safety visit, to ensure BP values have not dropped to unsafe levels (90 mm Hg systolic). Subsequent safety visits (up to 3) will be scheduled in two week intervals at the study physician's discretion. Blood pressure, heart rate, weight, adverse events (AE) and serious adverse events (SAE) screening, pill count (to assess compliance), metabolic blood panel and/or CBC, use of other medications data will be collected. Participants will return at Month 4 for a blood draw (metabolic blood panel and/or CBC), pill count and medication dispensing Participants will return again at month 8 for a second lumbar puncture, MRI, blood draw vascular ultrasound, ambulatory BP monitoring (optional) and cognitive testing. All aspects of the trial will be overseen by a data safety and monitoring board (DSMB) comprised of 2 physicians and a biostatistician not involved in the trial. Specifics of the procedures are detailed below.

7.2.1 Screening: Initial eligibility will be determined via phone interview by a member of the HEART team. When an individual responds to our recruitment activity by phone, a screening consent, I/E eligibility questionnaire and a Dementia Questionnaire regarding their family member will be obtained. This screening

process will determine the specific inclusion and exclusion criteria that can be evaluated by self-report, including age, hypertension or blood pressure diagnosis, and prior medical issues detailed in the inclusion/exclusion criteria. Those who are eligible via phone interview will be invited for a clinic screening visit along with their next-of-kin, if possible. The screening visit will include: explaining study details and obtaining informed consent, medical history, current medications, 2 seated blood pressure measurements, and baseline metabolic panel and/or CBC. Eligible participants will be asked to return for baseline evaluation and randomization.

7.2.2 Consenting Procedure: A signed consent form will be obtained from each participant. A single informed consent form will describe both the screening and study procedures. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant and will be documented in the participant's study record.

7.2.3 Enrollment, Baseline, and Randomization: During this phase, blood pressure measurements, detailed neuropsychological assessments, physical exam, brain MRI, and lumbar puncture will be completed. Blood samples will also be collected for APOE, inflammatory and endothelial markers and RAS functioning. Randomization and study intervention will occur after baseline data collection. Participants will then be started on the study medications, which they will be instructed to begin that evening. All study medication questions will be answered by study physician or nurse.

Follow-up Visits: Following the Baseline (randomization) visit, participants will be seen at two weeks. Blood pressure, heart rate, weight, adverse events (AE) and serious adverse events (SAE), pill count (to assess compliance), and use of non-study medications data maybe collected at some or all of these safety visits. New medication will also be dispensed during these visits. If blood pressure levels indicate hypotension (90 mm Hg systolic) we will contact the participant's PCP during that study visit and request treatment modification for the duration of the trial. As we are administering the lowest doses possible for this drug and because only 37% of individuals on an antihypertensive actually achieve proper BP control – we do not foresee hypotension being a common occurrence. The initial starting clinical does for telmisartan is 40mg once daily, which is considered our 'high' dose. Another 1/3 of the participants will be given the 'low dose,' 20 mg once daily and the third group is placebo. While 20mg is **lower** than the clinically recommended starting dose, this mechanistic study aims to assess the brain permeability of the active medication and thus higher doses are not necessary. If all measures are within normal limits, the participant will return at Month 4. If any NP or blood levels are NOT within normal limits, we will contact the participant's PCP and the participant will be advised to schedule a visit with their PCP for treatment. The participants will then be asked to return in 2 weeks for another safety visit.

Completion/Final Evaluation: The final visit (8-month visit) will include: blood pressure, heart rate, weight, adverse events (AE) and serious adverse events (SAE), pill count (to assess compliance), use of non-study medications, neuropsychological testing, neuroimaging, vascular ultrasound, lumbar puncture and blood collection. This visit will be identical to the Baseline visit except for randomization and medication dispensing. Participants will fast 8 hours prior to this visit.

8. SAFETY ASSESSMENTS

All data derived from this study is for research purposes only. Subjects will be monitored at all times for adverse events.

Potential Risks

Risk of Study Drug: The study will utilize a medication (Telmisartan), approved by the Food and Drug Administration for the treatment of hypertension and Cardiovascular Risk Reduction. It is well tolerated by young and elderly individuals and has been used extensively in clinical practice. Possible side effects include: dizziness, weakness, fatigue, lower extremity edema and hypotension. Renal failure and hyperkalemia are also potential complications. Adverse events will be queried at each visit. Alternatives to antihypertensive medications include no therapy, which is not safe or ethical in patients with hypertension. If there is

contraindication to telmisartan and another of the participant's medications then he/she will not be able to enroll in the study. Use of lithium is a contraindication telmisartan and hence lithium users are excluded. Aliskiren cannot be used in combination with telmisartan, which is why diabetes or use of any diabetes medication is exclusion. Use of drugs that act on the renin-angiotensin system during the second and third trimesters of pregnancy reduces fetal renal function and increases fetal and neonatal morbidity and death and thus women who are pregnant or planning to become pregnant will not be able to participate in this study.

J.1 Risk of Ambulatory BP Monitoring: Thought the BP monitor is lightweight and noninvasive, it can cause discomfort due to the repeated BP readings via tightening of the BP cuff. The most common risk pertaining to 24 hour BP monitoring is a skin rash. Participants with extremely sensitive skin or who have had prior reactions to BP monitors or skin patches are advised that this is a possibility in the current study, and they should consider this before opting to enroll. Participants will be advised to simply remove the BP cuff and turn off the monitor, should the discomfort become significant. All parts of the BP monitors are latex free and will be washed before use with nonabrasive, antibacterial soap.

J.2 Blood Vessel Function Measures: The vascular ultrasound is painless and is non-invasive and will be performed by the PI or a trained member of the HEART Project. We will place a pencil-like sensor gently against the inside the elbow and record a blood pressure signal from the subjects pulse. Dr. Wharton has used this procedure in her prior trials and is familiar with the data processing.

J.3 Risks of MRI: Subjects will have their MRI performed at the Wesley Woods Health Center 1841 Clifton Road Atlanta, GA 30329. Subjects with the following should not participate in MRI studies: metallic implants, such as prostheses, shrapnel, or aneurysm clips, or persons with electronic implants, such as cardiac pacemakers or claustrophobic individuals. The magnetic field generated by the MRI machine can cause a displacement or malfunctioning of these devices. There are no known risks or adverse effects from the radio signals used in this study. Also, subjects who have anxiety or claustrophobia will not be recommended to participate since the head must be placed fully inside the MRI scanner tube. In addition, fatigue and physical discomfort due to the length of the scan session are possible. The MRI scanner makes a great deal of high-pitched "beeping" noises during the scanning. To minimize this risk, we offer participants earplugs.

J.4 Risks of Cognitive Testing: In general, participation in cognitive testing is well tolerated by the majority of participants. However, if necessary, breaks in testing will be provided to those feeling either undue stress or frustrations during administration of tests.

J.5 Risk of Blood Draw: We will collect a blood sample at all main visits (Baseline, Month 4 and Month 8) as well as screening and safety visits. Blood draws can cause mild pain in the arm and may cause bruising, infection, and occasional fainting.

J.6 Risks of Lumbar Puncture (LP): An experienced member of the Emory ADRC and HEART Project will perform LPs for the study. The most common complication of a lumbar puncture is post-dural puncture headache. Studies show that the rate of headache occurrence can be reduced to as low as 1.7% with use of small needles (21-gauge or less), most commonly, we use a smaller 25-gauge Sprotte spinal needle. All subjects will be counseled on the signs and symptoms of post-dural headache and given information on management (ibuprofen, caffeine, and rest) and instructed to promptly contact the on-call study physician. A blood patch procedure by an anesthesiologist will be available to participants experiencing serious post-dural headaches. Bacterial meningitis is a very rare complication of lumbar puncture, occurring in less than 0.2%. Uncal or tonsillar herniation is a very uncommon complication, occurring in <1% of very high-risk subjects with known primary or metastatic neoplasms. Therefore, we do not anticipate such significant complications during the course of this study. A member of the ADRC research team and the current HEART Project trained in these procedures will perform a neurologic and fundoscopic exam on each subject prior to lumbar puncture and those participants thought to have papilledema or gross neurologic deficits consistent with a central nervous system mass will not have a lumbar puncture performed. Each subject will be called the day following his or her lumbar puncture to ask about any side effects. All subjects will be given a telephone number to reach a physician 24-hours a day.

J.7 Social: The social risk is a breach of confidentiality regarding risk for development of AD. Subject names and research data will be handled with utmost confidentiality and discretion. Subjects will be assigned a unique identification number that can be traced only by the research coordinator and Principal Investigator. All subject information will be kept in locked drawers, file cabinets, or secure computer files, with access only allowed to research personnel.

J.8 Ethical, Psychological, Legal, Other: Although participants will be given information on their circadian BP levels at the conclusion of their participation, they will not be given information about their APOE4 status, CSF or plasma biomarkers. This will be clearly stated in the informed consent process. Although ApoE4 allele is associated with a higher risk of developing AD, not all people with ApoE4 develop AD and not all AD patients are ApoE4 allele carriers. Also, there are no clear standards in cognitively healthy adults for identifying higher risk individuals using CSF or plasma biomarkers. Furthermore, although these markers *may* predict who is at higher risk of developing AD, they are still in the research stages and do not have proven roles in clinical practice. In addition, since there are no established effective preventive therapies for AD, providing individuals with individual risk data may only add to their psychological burden and not provide them any benefit. If, during the course of the study, a new medication is approved by the FDA for AD prevention in high-risk individuals, then current policies will be revised to ensure good clinical care for study participants. Insurability of participants may be affected if there is any breach of confidentiality, which we do not anticipate.

J.9 Protection of Subjects: As stated above, the physical, social, and psychological risks associated with this study are small. Confidentiality will be assured by assigning a unique subject number to each participant. All subject information will be kept in locked drawers, file cabinets, or secure computer files, with access only allowed to the HEART research personnel.

J.10 Risks and expected benefits for subjects and society: Although individual participants will likely not derive any benefit from participation, they may benefit from future research that builds on these data should BP control eventually be proven to have a role in AD prevention. The benefits to society may be considerable. Society, specifically individuals at risk for AD, will benefit by learning the extent to which AD biomarkers markers indicating risk for cognitive decline are related to vascular risk factors and blood pressure fluctuations. This is not a treatment study and there are thus no alternatives to participation.

Data Safety and Monitoring

Safety procedures will be implemented in accordance with NIH safety policies for clinical trials. The safety monitoring will be conducted by the PI and a Data and Safety Monitoring Board (DSMB) on an annual basis. The DSMB is comprised of 3 members including at least 1 physician familiar with RCTs and African American HTN studies, a researcher whose area of expertise aligns with the primary outcome of the study and an experienced biostatistician. Once an SAE has been identified, it will be reported to the Emory-IRB. The Alzheimer's Association (the funding organization) will also be notified. The DSMB will be asked to review the event. Once a year, the DSMB will review progress of the trial (recruitment and follow-up, protocol violations), assess the safety of the protocol, and address any issue that limits the study success. Instructions and a template for documents for the DSMB (i.e., ICF, source documents, notes to file, protocol deviations, AEs) will be provided to the DSMB in advance of their annual review. The report will include all AE and SAEs to be reviewed by the DSMB. If concerns arise about safety issues, the DSMB may request additional data and propose specific analyses. A report summarizing the review, discussions, and recommendations for continuation of the trial will be generated and signed by DSMB members and provided to the IRB.

8.4 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

At each encounter, standardized adverse event (AE) monitoring forms will be completed. At any point during the study period an adverse event is identified, it will be recorded in the participant research record along with the time/place of the event. The study physician will be immediately notified and the participant evaluated either by phone (if participant is not on site) or face-to-face (if participant is on site). If the AE is related to

anticipated AE lumbar puncture headache, the SOP for Lumbar Puncture headache AEs will be followed and the information will be reported to the study physician. Depending on the physician assessment, further evaluation maybe performed such as further lab work, exam, or referral to the emergency department. Details about the safety parameters will be recorded and entered into a database. Comparisons between the 3 groups will be conducted to assess overall and between group differential safety.

8.5 Adverse Events and Serious Adverse Events

During the screening process, if the participant is identified to have a problem requiring medical attention such as severely elevated blood pressure, they are referred to their primary care physician or the emergency room. The primary care physician will be notified of all screening results by mail or fax upon the participant request. Adverse events will be reported to the Emory IRB, the DSMB, the funding agency, and the primary care provider of the participant. If the participant does not have a primary care provider, they will be referred to one. The timing of reporting depends on whether the adverse event is expected, is serious or not, and whether it suggests that other participants are at higher risk of harm. Any subject who develops a serious adverse event (SAE) requiring emergency medical care during the study visit will be given immediate medical care at the local hospital by the medical investigators, intervention will be stopped and the participant will be referred back to their primary care physician for ongoing hypertension care. SAEs will be reported to the above-named agencies within 24 hours of their discovery. Other adverse events (non-serious) will be reported within 2 weeks of their discovery.

8.6 Incidental findings

Incidental blood chemistry abnormalities, such as elevated blood sugar, will be reported to the subject and primary care provider. Incidental findings on brain imaging such as brain masses, prior stroke, and hemangiomas, will be evaluated by the PI and the study physician and relayed to the subject and her/his provider. In the unlikely event that an incidental finding requires immediate attention such as bleeding on MRI, urgent care will be provided in a facility of the participant's preference.

8.7 Reporting Procedures

Expected side effects described within the safety parameters will be reported to the IRB, DSMB and NIA as part of ongoing progress. SAEs will be reported to the DSMB, the Alzheimer’s Association and IRB within 24h. A detailed report will follow within 7 days, and then a final resolution report will be sent. Other causes or existing health issues will be investigated and ruled out as the inciting factor prior to the determination of an adverse reaction.

8.8 Follow-up for Adverse Events

With the permission from the participant, we will contact the participant’s primary care doctor and help him/her get medical follow-up. Adverse events that require follow-up such as increased potassium or increased creatinine will be monitored until resolved or are further followed by the primary care provider. If emergency care or hospitalization was necessary, the study physician will conduct a follow-up phone or face-to-face evaluation. If the event requires intervention discontinuation, the participant will be referred back to the primary care provider. They will also be invited to perform the final visit evaluation including all study procedures planned for that visit.

9. INTERVENTION DISCONTINUATION

A subject may choose to withdraw from the study for any reason. The investigators may also request that the subject withdraw from the study, for safety or other reasons. Withdrawn participants will continue to be monitored by the study personnel after the event if possible and invited to complete the final visit evaluation. The criteria for discontinuing a subject’s participation include:

(1) the subject's request,
(2) serious adverse events that requires un-blinding,
(3) new stroke, transient ischemic attack, or myocardial infarction that limits ability to complete the study
(4) Symptomatic dysrhythmia
(4) anaphylaxis or allergic reaction to study medications
(5) inability to participate due to relocation or other personal reasons
(6) renal failure (increase in serum Creatinine above 2.5 mg/dl) or hyperkalemia (greater than 5.1 meq)
(7) pregnancy or planning to become pregnant

11. DATA COLLECTION AND QUALITY ASSURANCE

11.1 Data Collection Forms

Data collected during interviews and exams will be documented on trial-specific data forms. Neuroimaging data will be saved in digital formats on a HIPAA-compliant server.

11.2 Data Management

Once a subject is enrolled into the study, he/she will be assigned a unique identifier number and be referred to by initials and the study number only. Only research team members will have access to the files. Data will be entered on a web-based secure trial data system; the data system will include must enter fields, range checks, and simple logic checks. The trial database will include for all variables an electronic data audit of data edits (who, when, and why). A data query report (including missing, out of range, and logic checks) will be generated by the trial statistician weekly; timeliness and completeness of responses to data queries will be monitored. Trial databases will be stored on a secure server. A copy of the master participant list will be kept by the PI in a locked office. The investigators will keep subjects' medical records private as far as the law allows. The IRB and officials of the sponsor/funding agency will have access to these records as needed within legal guidelines. If study results are published in journals or presented at meetings, we will not use the subjects' name. All investigators and research team members have successfully completed the online CITI program for working with Human Subjects in Research.

11.3 Quality Assurance

11.3.1 Training: Research personnel will be trained by the PI and the ADRC investigators and staff. The process of training consists of data forms completion, neuropsychological assessment, data storage and human subjects protection, and vital sign evaluation will be documented in a training log for each study personnel.

11.3.2 Quality Control: To assess quality of data collected the PI along with another investigator will randomly and on intervals review data obtained. In addition, quarterly assessment of personnel competencies in obtaining data (e.g. Blood pressure checks, neuropsychological assessments etc.) will be performed.

11.3.3 Protocol Deviations: Every attempt will be made to maintain compliance with the approved study protocol. In the unanticipated event when a deviation is noted, the Emory IRB will be notified. The PI will conduct an investigation about the setting, reasons and potential remedies that need to be instituted to rectify the deviation and prevent future similar instances. The DSMB will also be notified.

K. Inclusion Report

Inclusion of Women: All participants will be invited through clinic visits, recruitment registry or individual flyers to participate in this study. Since approximately 70% of AD cases are women, we expect that percentage to be reflected in our study population and we will emphasize the importance of AD prevention research in the context of women's health to those invited to participate.

Inclusion of Minorities: Self-reported African American status is an inclusion criteria for this Phase I RCT, thus all participants would be classified as 'minorities.'

L.1 Adequacy of Protection against Risks

Recruitment and Informed Consent

To ensure that participants have adequate time to review participation in the study and discuss the risks with their friends and family, we will email a blank ICF to each participant prior to their Screening visit. While this does not in any way take the place of the in-person consenting process, this extra step allows participants to grasp the details of the study visits and have time to formulate questions to ask the coordinator during the visit. Dr. Wharton has employed this procedure in her ongoing and prior trials and her participants have responded very favorably to the additional time to peruse the study specifics.
The actual consent process is conducted as a face-to-face interview with the participant during their screening visit. No study procedures are initiated until the consent process is complete. After all questions have been answered by a study team member, the participant signs the consent form and they are given a copy for their records.

L.2 Potential Benefit to Subjects:
There are no direct benefits from participation in this research study.

L.3 Importance of the knowledge to be gained:
The information gathered in this study may be of benefit to persons with AD in the future. The scientific data gathered in this study may possibly provide a better understanding of the brain regions that are affected in AD, which may consequently lead to methods for earlier detection and treatment monitoring. Furthermore, scientific data from this study may improve prediction of incident AD. Currently there is no cure or effective treatment for AD, though several promising drugs are under investigation. As better treatments become available, it will be important to identify candidates for treatment as early as possible, and to develop methods to monitor treatment effects on the brain. Methods for examining the brain using MRI combined with CSF biomarkers may greatly facilitate detection and observation of preclinical AD.

L.4. Compensation
Participants will be compensated a total of $150 for their participation. $75 will be given at the conclusion of the baseline visit and the remaining $75 after the conclusion of the Month 8 visit. Compensation will be given in the form of gift cards.

5. 5. Analyses and Power Calculations: John Hanfelt, PhD **(Key Person)**, ADRC Data Core Co-Leader will oversee data analyses for the proposed trial. We will conduct Wilcoxon-Mann-Whitney tests to assess whether the change in the level of each CSF biomarker (month 8 vs baseline) differs between the treated group and placebo group. Tests will be conducted separately for each dose group (20mg and 40mg) but we will also consider testing for a dose-response by conducting Jonckheere-Terpstra trend tests. P-values < 0.05 will be regarded as significant. We will consider linear regression analysis (outcome is 8-month change in a biomarker) to adjust for demographic or clinical confounders. Power calculations for ACE, CBF, and Aβ comparisons are based on data from our previous trials in similar populations. With 20 participants per group, will have 99% power to detect change in ACE levels and 80% power to detect differences in CSF Aβ and CBF between the treated groups and placebo (based on 2-sided tests and Type I error rate $\alpha = 0.05$).

5. 6. Relationship to Future Grants: Data from the proposed trial will be used in conjunction with Dr. Wharton's prior studies for POC regarding CNS penetration, dose effects, and data for power calculations for an NIA R01 Phase IV application to be submitted in 2018, examining brain RAS function, Aβ and RAS acting medications longitudinally, in normotensive and hypertensive African Americans and Caucasians at-risk for AD.

Data Safety Monitoring Board Committee (DSMB):

Our DSMB consists of the following members.

Donald Bliwise, PhD
Professor of Neurology (primary appointment)
Professor of Psychiatry and Behavioral Sciences (secondary appointment)
Emory University School of Medicine
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