Title: Endothelial Facilitation in Alzheimer's Disease; An open-label pilot study of the sequential and cumulative effects of Simvastatin, L-Arginine, and Sapropterin (Kuvan) on cerebral blood flow and cognitive function in patients with Alzheimer's Disease

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INSTRUCTIONS:

1) **Title**

Endothelial Facilitation in Alzheimer's Disease; An open-label pilot study of the sequential and cumulative effects of Simvastatin, L-Arginine, and Sapropterin (Kuvan) on cerebral blood flow and cognitive function in patients with Alzheimer's Disease.

2) **IRB Review History***

N/A

3) **Objectives***

Purpose and hypothesis: The purpose of this study is to determine whether treatment of subjects who have mild Alzheimer's disease or Mild Cognitive Impairment (MCI), using a combination of drugs that increase endothelial nitric oxide synthase (eNOS), can increase cerebral blood flow, and improve cognitive function.

Alzheimer's Disease (AD) is a degenerative disorder of unknown etiology, characterized by progressive dementia, and hallmark pathology at postmortem of senile plaques and neurofibrillary tangles in the brain. It may be caused by rare (3%) dominant genetic mutations (APP, Presenilin 1 and 2), occurring as an Early Onset Dominantly Inherited (EODI) form; but more commonly occurs as Late Onset Sporadic AD (LOSAD), which is sporadic, but may be associated with more common genetic risk factors (APOEe4). Age is by far the most important risk factor, however; and vascular risk factors (diabetes, hypertension, dyslipidemia) are strongly related to the incidence of AD. While much research has been devoted to the "amyloid hypothesis," that beta amyloid (Aβ) formation is the underlying cause of AD, it remains uncertain whether the Aβ is actually the cause of AD, or a secondary consequence, and a "marker" as AD develops. Aβ is found in brains during normal aging; and clinical trials of drugs that block the formation of Aβ, or remove it, have not produced a benefit; nor has the amount of brain Aβ - determined during life, or at post-mortem - been shown to relate to the severity of dementia.

Decreased cerebral blood flow is characteristic of AD; and we and others have shown that brain microvascular endothelium is scanty and poorly stained immunohistochemically. We and others have shown that statin drugs reduce the incidence of AD in individuals treated with statins, independent of cholesterol levels. The microvascular endothelium is known to be a paracrine organ with important trophic secretory activity, critical to maintaining the normal function and growth of cells, including neurons, and the healthy maintenance of organs. Microvascular capillaries lie in direct proximity to every one of the hundred billion neurons in the brain, and we hypothesize that their endothelial trophic secretions, as well as blood supply to neurons, are critical to the survival and function of the brain.

The hypothesis being tested is that improving brain microvascular endothelial function will help to maintain the normal, healthy function of the brain, and avoid atrophic changes with advancing age that often result in cognitive decline and eventually may cause Alzheimer's dementia. We propose continued study of an open label clinical trial consisting of a series of synergistic treatments that are known to improve endothelial function. The treatments will be used in subjects with early AD and MCI.

Three drugs – all FDA approved – improve endothelial eNOS in a synergistic, interactive manner- simvastatin, L-Arginine, and sapropterin (Kuvan). As a surrogate measure of microvascular endothelial function, and efficacy of the treatment, we will measure cerebral blood flow (CBF) using MRI-based arterial spin labeling, and contrast perfusion imaging techniques; and will assess cognitive function, which should either improve (although more slowly), or evidence a decreased rate of cognitive decline over time, compared with the usual progression of AD.

4) **Background***

At present, the etiology of LOSAD remains unknown (1-4). Characterized by gradual-onset, progressive dementia, its hallmark cerebral pathology is defined by neuritic plaques containing fragmented neuritic particles and β–amyloid (Aβ), and by neurofibrillary tangles (NFT), containing paired helical filaments comprised of hyperphosphorylated tau protein. Loss of synapses (5-7) and neurons (5, 7), and increasing cortical atrophy (8) underlie the declining cognitive function. These changes often, but not always, begin in the medial temporal lobe structures, including the entorhinal cortex and the hippocampal areas. While this pathology is indistinguishable from that seen in the rare, EODI AD, which appears to be caused by mutations of genes related to the formation of Aβ (3, 9) (as well as to Notch1 activation (9)), it is not clear whether Aβ, or its precursor protein (APP), have a primary causal role in the development of neural degeneration and dementia in EODI AD. The relation of amyloid to LOSAD is even less clear. As one indication of this uncertainty, therapeutic efforts to remove amyloid in humans (e.g., by immunologic means), or to interfere with its production by blockade of the key beta and gamma secretases that cleave Aβ from its parent protein –APP-, have so far been ineffective in modifying AD (10, 11); nor is the amount of Aβ imaged by PET scanning with Pittsburgh Compound B (C-PIB) clearly related to cognitive impairment (12). Despite the evidence that Aβ dimers can be toxic to synapses (13), there is increasing doubt regarding the hypothesized principal role of Aβ as the fundamental etiology of sporadic AD, suggesting that the accumulation of amyloid may be a downstream effect (14), or a marker of the underlying degenerative process.

What then are the probable mechanisms of development of AD? The strongest *risk factor* for LOSAD, which comprises more than 95% of AD, is, by far, advancing age (2). The risk of developing AD doubles every 5 years after age 65 (15), with almost 50% of individuals showing significant cognitive

impairment by age 85. The annual incidence of AD at 85 is 20-30 times greater than at 65 (16). Other identified risk factors include the presence of an apolipoprotein Eε4 allele; and importantly, a number of *vascular risk factors*, which include hypertension, diabetes, and dyslipidemia (17-20).

In human studies, analyses performed with PET and SPECT scanning have shown decreased metabolism and CBF in patients with AD, particularly in the parietal regions, and involving frontal and temporal areas as well (21, 22). Microscopic examination of post-mortem AD brain with specific immunohistopathologic stains for microvascular endothelium (CD 31) reveals narrowed, tortuous microvessels with scanty, poorly-stained endothelium in affected AD cortical tissue. While the possibility exists that these vascular changes may be a consequence of altered brain structure, or of diminished functional demand, the consistent epidemiologic association of multiple vascular risk factors with the incidence of AD suggests that the vascular changes are a plausible causal factor, initiating or contributing to the pathogenesis and development of AD.

One line of reasoning suggests that the failure of brain microvasculature may have an important role in the pathogenesis of AD. A number of studies support the concept that the microvascular endothelium in brain (and elsewhere) is a major paracrine tissue, secreting trophic factors that are critical in maintaining organ/tissue integrity (23-26). This concept, pioneered by Judah Folkman (27), underlies an important strategy in the therapy of cancers, in which tumor tissue deprived of adequate vasculature has been shown to shrink. Angiostatin agents, which block angiogenesis and the integrity and function of vascular endothelium, have been used to control the growth of a number of experimental and human cancers. Folkman also noted, however, that in experimental animals, transplanted *normal* tissue will not survive and grow when vascular function is blocked by angiostatin agents, and will undergo atrophy. Whether this is due to inadequate blood flow (the conduit function of blood vessels), or to the paracrine functions of endothelium, is not known. Factors secreted by endothelial cells in cell culture have been shown, for example, to be critical for the survival, growth and differentiation of neural stem cells, emphasizing the importance of these trophic influences in the maintenance and growth of normal neural tissue (26).

Early in the course of AD – as an initial event – synaptic, axonal, then neuronal loss, with atrophy of brain in affected areas, takes place. While decline of many critical molecular housekeeping functions may contribute to this involutional atrophic change, which eventually evolves into major, widespread cortical atrophy, degeneration and dementia, we hypothesize that an important component initiating this degenerative cascade and atrophy is the loss of microvascular trophic and nutritional functions. The goal of the present therapeutic intervention, therefore, is the facilitation of optimal brain microvascular endothelial function. While direct monitoring of trophic functions of the endothelium is not practical, microvascular integrity and function can be assessed by measuring changes in CBF; the paracrine and microvascular flow

functions are parallel, related functions. Assessing cognitive function should also provide information on the important beneficial effects on brain function.

Endothelial Targets:

Endothelial function can be facilitated by increasing eNOS activity, and by reducing endothelin-1.

Endothelial nitric oxide synthase, or Type 3 nitric oxide synthase, synthesizes NO from L-arginine, which then facilitates vascular relaxation via cGMP. eNOS activity can be increased by the administration of HMGCoA reductase inhibitors (statins), which are widely used to reduce cholesterol synthesis by blocking the mevalonate pathway. Statins have pleiotropic effects, however, which are known to reduce the risk of vascular events well beyond the reduction of total cholesterol, or of LDL. The effects of statins on eNOS (28, 29), and on endothelin-1 (30), as well as their anti-inflammatory effects, are believed to be instrumental in its efficacy.

Production of NO can be enhanced by the administration of its precursor amino acid, L-arginine (31). The synthesis of NO also requires a critical cofactor, tetrahydrobiopterin (BH4; sapropterin; Kuvan) (32). Thus, the opportunity to enhance brain eNOS activity by the stepwise addition of statins, L-Arginine and BH4 (Kuvan) could be utilized to counteract age-, environmental- and genetic factors impairing cerebral microvascular function, and enhancing the trophic and nutritional influences of microvascular function in the brain.

The goal of this therapeutic study is the stepwise enhancement of eNOS activity in order to improve microvascular endothelial function in the brains of subjects with early stages of AD and MCI.

Investigator's background related to Alzheimer's disease

David Drachman has been involved in research on memory, the brain and Alzheimer's Disease for most of his long career in academic Neurology. His work on herpes simplex encephalitis confirmed the critical role of the hippocampal complex in memory (34), and initiated his interest in the cognitive deficits of aging. His seminal work demonstrating the role of the cholinergic system in memory, and the observation that cholinergic blockade with scopolamine in young subjects reproduced cognitive deficits found in aging and dementia (35, 36), resulted in the development of 3 of the 4 drugs (the anticholinesterases) currently approved for the treatment of AD. He co-authored the NINCDS-ADRDA criteria for AD (37) – which is the most-cited Neurologic reference in the medical literature. His laboratory first demonstrated the loss of synapses in AD (6), and he was a collaborator in the studies that first demonstrated the genetic basis of Familial AD (38). He served as Chairman of the Medical and Scientific Advisory Board of the National Alzheimer Association, and was a member of the FDA panel on neurological drugs for four years. He participated in clinical trials of Aricept, Namenda, Lipitor and other drugs for the treatment of Alzheimer's Disease. He has developed a screening test for dementia (39), and for the behavioral disorders occurring with Alzheimer's Disease (40). He was the senior

author on a seminal study of the role of statins in preventing dementia and AD (41). Over the years, he has written extensively on the etiology and mechanism of Alzheimer's Disease (2, 42-46).

Dr. David Drachman was the mentor for Dr. Elizabeth DeGrush during their shared time at UMASS in the neurology department. Unfortunately he has since passed away, prior to completion of the study. Prior to his death, he chose to assign her the Primary Investigator role for the continuation of the study.

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Preliminary Data

Ten subjects have completed the 6 month treatment program. From preliminary analysis of the first two subjects in the study, MRI-based arterial spin labeling and gadolinium perfusion studies reveal a significant degree of increase in CBF from baseline to the conclusion of the 6month period. The psychometric studies reveal moderate improvement in psychometric test performance, and subjective measures of cognition and performance of activities of daily living (CDR; CIBIC Plus) indicated by the experimental subjects, their spouses, and family members reflect evidence of improvement. The remainder of the data have yet to be analyzed. There were no adverse events attributable to the medications used.

5) **Inclusion and Exclusion Criteria***

Inclusion Criteria: Subjects must have mild AD and MCI; age between 55-85; Mini Mental State Exam (MMSE) between 15-26; a caregiver who can provide information, and bring patient to the sessions; no known allergies to any of the medications to be used; normal renal function; willingness of patient and spouse/responsible caregiver to participate.

Exclusion Criteria: Significant Psychiatric disorder; stroke; current use of any of the test medications (e.g., statin, L-Arginine, Kuvan); phenylketonuria (PKU) ; elevated serum phenylalanine level (>10 mg/dL); allergy to any of the medications; current active malignancy; renal insufficiency (elevated creatinine above 1.3mg/dl); abnormal liver function (ALT or AST 2x normal); other serious disease including coronary insufficiency or congestive heart failure, carotid stenosis greater than 50%, active peptic ulcer, urinary tract or other active infection, cancer (except skin cancer, or 5 years inactive breast or prostate cancer) etc.; pregnancy; or inability to come to UMass for follow-up. Subjects may continue to take anticholinesterase drugs for AD (Aricept, Exelon, Razadyne) and/or Namenda, if they have been on the drug(s) for at least 3 months. Subjects on levodopa and male subjects taking drugs for erectile dysfunction (Viagra, Cialis, Levitra) are cautioned regarding hypotension.

Adults unable to consent: All of the subjects have some cognitive impairment, but most will be able to provide informed consent. Regardless, we require that a responsible family member also responds regarding the patient's inclusion in the study, after appropriate information and the consent form have been presented.

No children or adolescents; no pregnant women; and no prisoners are to be considered for inclusion in this study of late-onset Alzheimer's disease.

6) **Study-Wide Number of Subjects***

N/A

7) **Study-Wide Recruitment Methods***

This is a single center study at UMass Medical School, and recruitment is under the control of the PI.

Subjects will be recruited from 6 sources:
1) UMass Neurology Clinic patients seen for early dementia and/or their legally-responsible caregivers, may be asked if they would be interested in participating in the study
2) An advertisement (see attached) will be sent to Daycare centers and Senior centers in the Worcester vicinity
3) The Massachusetts/New Hampshire Alzheimer Association has been notified of the availability of the study, and asked to publicize it.
4) Word of mouth among patients in the Neurology Clinic at UMass and affiliated hospitals, and physicians will continue to be used to publicize the availability of the study.
5) Newspaper advertisements in local newspapers in the Worcester vicinity will indicate the availability of the study.
6) The trial is listed in clinicaltrials.gov, and family members or patients can respond to that listing

8) Study Timelines*

- Each subject will be enrolled for 4 months after baseline testing. In the case that significant improvement is noted, this may be extended for compassionate use. In the case that any significant adverse effects are observed, the study will be terminated before the end of the 4-month interval.
- We anticipate that it will require between 2 and 3 years to enroll all ten subjects
- The primary analyses should be completed by June, 2015

9) Study Endpoints*

The primary endpoint of the study is the increase of CBF resulting from the combination of 3 experimental drugs, comparing the CBF at the end of the study with the baseline determination. Since this is a pilot study, global CBF and focal CBF over several critical areas (e.g., hippocampus; parietal lobes, frontal lobes) will be compared. The arterial spin labeling CBF and the Gadolinium-based perfusion CBF will both be examined for this purpose.
The secondary endpoint will be the performance of the subjects on the psychometric tests done at each return visit and at the end of the 4- month study, in comparison with their performance at baseline. This includes the MMSE; Alzheimer's Disease Assessment Scale - Cognitive (ADAS-Cog); Cognitive Assessment Screening Test (CAST); the Clinical Dementia Rating (CDR/sum of boxes); and the Clinician Interview-Based Impression of Change plus caregiver input (CIBIC plus).

The safety endpoints include any significant adverse symptoms; abnormal observations on physical examination that occur during the study; and any

significant adverse changes in the laboratory studies done at monthly and end-of-study testing (CBC, metabolic profile, lipid profile, CPK, UA; EKG).

10) **Procedures Involved***

1) Baseline evaluation:

a) Neurological Examination; general physical examination; CAST.

b) Laboratory studies: CBC/differential, ESR; metabolic profile, CRP; lipid profile, CPK; FTI/TSH, antithyroid Ab's; RPR; B12 level; HbA1C; ANA; PT/INR; urinalysis.

c) Imaging: MRI brain, MRA brain and neck These studies will be done at a single session in the MRI suite. The MRI and MRA are done without moving the patient by using different settings on the MRI scanner. The MRA requires the intravenous injection of Gadolinium, as described. Subjects who are claustrophobic may be given 0.5- 1.0 mg. Klonopin, a tranquillizer, to prevent anxiety during the scanning procedure. ,

d) Cerebral blood flow (CBF). is done in the advanced MRI facility, before drug treatments on subjects who have no exclusion criteria, and choose to proceed in the study. Klonopin 0.5-1.0 mg may be given to prevent anxiety before the scan.

e) Psychometric testing- MMSE; ADAS Cog; CDR/sum of boxes; CAST

f) ECG

Abbreviations: All the following are blood tests: CBC- Complete Blood Count; **ESR-** Erythrocyte Sedimentation Rate; **CRP-** C-Reactive protein; **CPK-** Creatine Phosphokinase; **FTI-** Free Thyroxine Index; **TSH-** Thyroid Stimulating Hormone; **Antithyroid Ab's-** Antithyroid Antibodies; **RPR-** Rapid Plasma Reagin; **B12-** Vitamin B12; **HbA1C-** Hemoglobin component A1C (to which glucose is bound); **ANA-** Anti-Nuclear Antibodies; **PT/INR-** Prothrombin Time/ International Normalized Ratio (for blood clotting).

The following is a cardiac test: EKG - Electrocardiogram

The following are imaging studies: MRI- Magnetic Resonance Imaging **(**of brain); **MRA-** Magnetic Resonance Angiography; CBF- Cerebral Blood Flow

The following are psychological tests: MMSE; **ADAS-Cog**; **CAST**.

Drug name: BH4 – Sapropterin or Kuvan

2) Drug administration:

a) Week 0
   a. Simvastatin 40 mg/day, at bedtime, for 30 days; continue; and add:

b) Week 4
   a. L-Arginine 2.0 gm TID and HS for 30 days; continue; and add:

c) Week 8-16
   a. Sapropterin (Kuvan; BH4) 20 mg/kg/day for 60 days;

Following taking of each drug, the following tests will be done. The next medication will be given to the patient/caregiver at this testing session, and they will be notified within 2 days (i.e., after the laboratory results are available) to begin taking the next drug if all laboratory tests are within acceptable normal limits.

i. Interview of caregiver and patient re adverse effects:
   Dizziness, headache, confusion, sleep disturbance, rash, cardiac symptoms, respiratory symptoms, GI symptoms, urinary symptoms; other (per informant)
ii. Laboratory studies: CBC, metabolic profile, lipid profile, CPK, UA,
iii. Cognitive function, memory (caregiver, patient) MMSE; CIBIC Plus (Clinician Interview-Based Impression of Change, Plus Caregiver information)
iv. Cerebral blood flow imaging study

3) At Week 16 (end of study)

a) Interview of caregiver and patient re adverse effects as above;
b) Laboratory studies: CBC, metabolic profile, lipid profile, CPK, UA; EKG
c) Cognitive function, memory (caregiver, patient)
   a. MMSE; ADAS Cog; CDR/sum of boxes; CIBIC Plus
d) Cerebral blood flow imaging study
e) Electrocardiogram
f) after completion of the 4- month study, subjects will have the choice of continuing to take the three study medications, or may opt to taper and discontinue them over 8-day periods for each drug, as described above (tetrahydrobiopterin first, L-Arginine second, simvastatin third). For subjects who choose to continue the drugs, they will be provided at no charge to the subjects until the last enrolled subject has completed the study, and will be tapered as described above. Subjects who choose to continue the medications will be followed at 3-month intervals in the Neurology Clinic at UMass, with appropriate studies to monitor safety, including a comprehensive metabolic profile, CPK, ESR and complete blood count. Note that since the drugs are FDA approved for other indications, they could be given “off-label.”

Procedures to lessen probability/magnitude of risks:

a) Patients are examined at baseline, and excluded if they have any significant medical or psychiatric disorder. Patients with cancer, renal insufficiency, stroke, myocardial infarction, phenylketonuria, abnormal liver function, coronary insufficiency, congestive heart failure, or intolerance to any of the drugs to be used are excluded.

b) All subjects must have a responsible caregiver available to observe them on a daily basis, and report any untoward changes in function to the PI or other investigator.

c) Baseline and repeated inquiry about medical problems, physical examination, and repetition of laboratory studies at each return visit to observe any adverse effects of drugs, or changes in medical status, are carried out.

Drugs and devices used in the research; purpose of their use; regulatory approval status

Three FDA-approved drugs are used, sequentially and cumulatively, for the purpose of increasing cerebral blood flow by facilitating brain endothelial nitric oxide synthase (eNOS). The drugs are: simvastatin; L-Arginine; and sapropterin (tetrahydrobiopterin). In addition, patients who have significant claustrophobia if in the MRI scanner may receive a small oral dose (0.5 to 1.0 mg.) of clonazepam.

Data Collection

Data regarding baseline physical and laboratory status, and the same data on each return visit will be collected and recorded in each subject's file.

Cerebral blood flow data will be collected from each evaluation.

Data regarding cognitive testing will be collected for each visit.

The formal follow-up of subjects is 4 months (120 days), during which the data, as described, are collected and recorded. Subjects are returned to their primary care physicians and managing neurologists. They are asked to return two months after conclusion of the formal study for brief neurological evaluation (not a part of the study); and may continue to be followed afterwards in the Neurology outpatient clinic at UMass at their choice

11) **Data and Specimen Banking***

No blood, spinal fluid or other specimens are stored for subjects in this study.

Data are stored electronically on a secure, HIPPA compliant UMASS server. Any paper copies of data or patient information are kept in a locked cabinet in a locked room. Data are available to the PI and other investigators involved in the study. Relevant medical information may be made available to the subjects' personal physicians in case of any adverse effects, either related to the use of the medications used in the study, or significant coincidental medical events observed during the course of the study. All data are secure; HIPAA rules apply to all medical information.

Data presented or published regarding the results of the study will be de-identified as to individual origin and subjects included.

12) **Data Management***

Power analysis: This is a pilot study involving ten subjects, and data are being collected for preliminary assessment of the potential benefits of the treatment, for consideration of conducting a larger scale study. As a result, no power analysis is planned for this pilot study.

Data analysis: The CBF and psychometric data obtained for each individual subject at baseline will be compared with any changes observed in comparable data obtained at each subsequent assessment (one month, two months, four months). The data will be analyzed to determine change: improvement, stability or decline; and to assess statistical significance of magnitude of change with addition of each added drug (and the conjoint administration of all three drugs) during the four-month course of the study.

Security: Data are stored electronically on a secure, HIPPA compliant UMASS server. Any paper copies of data or patient information are kept in a locked cabinet in a locked room.. All investigators must pass the CITI examination. Access to the data is restricted to the investigators who handle the data, which are confidential. As this is a pilot study, and treatment is not blinded, subjects will be identified by their individual names and UMass medical record numbers for the qualified investigators in the study. All reports will use sequential coded numerical designations for subjects.

Data storage: Data will be stored for the duration of the study, and 5 additional years. Each investigator is responsible for transmission of the data collected by her/him; i.e., psychometric data by the investigator administering the test; cerebral blood flow by the imager, and the data analyst recording and analyzing the data, etc.

Collected data include all medical, clinical laboratory, psychometric, imaging and cerebral blood flow data. All these data are maintainedelectronically on a secure, HIPPA compliant UMASS server.. In addition, routine laboratory data (blood studies, urine, EKG) will be entered in the UMass Allscripts record in order to be available to the subjects' primary care physicians.

13) **Provisions to Monitor the Data to Ensure the Safety of Subjects***

All clinical and clinical laboratory data are collected and recorded at baseline and at each visit (4 weeks, 8 weeks, 16 weeks). All the data are reviewed by the PI for each subject following each visit. Report forms noting the results of the tests are available for each visit. In addition, each subject's family member has a direct telephone number to notify the PI of any adverse event, whether related or not to the medications.

All adverse events related to the study drugs are reported to the UMass IRB, and the FDA. All KUVAN™ serious adverse events, whether or not considered drug-related, will be reported and documented on Form FDA 3500 A

(MedWatch Form) and forwarded directly to BioMarin Pharmaceutical Inc. Drug Safety Department within 24 hours (Contact information on file). This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences.

14) **Withdrawal of Subjects***

Subjects may be withdrawn without their consent under the following circumstances:

I. The investigator decides that continuing in the study would be harmful to them.
2. They need treatment not allowed on this study.
3. They fail to keep your appointments or take the medications as instructed.
4. They become pregnant.
5. The study is canceled by the investigators or the UMMS Institutional Review Board.

The subject/caregiver is asked to come to the University of Massachusetts Medical Center to meet with the PI. Laboratory studies, as scheduled for the final visit, will be carried out to assess the absence of any significant adverse effects. In the case that any significant adverse effects occur, the study will be terminated before the end of the 4-month interval by tapering the doses of the drug causing the adverse effect over an 8-day interval. This will be done by giving ¾ of the maximum dose for 2 days; then ½ the max. dose for 2 days; ¼ the max dose for 2 days; then 1/8 the max dose for 2 days, then discontinuing it. If the subject is on all 3 drugs, tetrahydrobiopterin will be tapered first; L-Arginine second; then simvastatin last. If only one or two of the drugs have been given, they will be tapered as described. In case of any severe adverse effects, the affecting drug may be discontinued immediately, as needed.

If the subject declines a final visit, the telephone contact with the responsible caregiver may comprise the final encounter.

15) **Risks to Subjects***

Risks are primarily related to the drugs used in the study:

Simvastatin can produce elevation of liver function tests in approximately 1% of patients, and myalgia in about 1-2%, with elevated creatine phosphokinase (CPK) in less than 2%. Rarely, rhabdomyolysis (muscle breakdown) may occur, evidenced by muscle pain, weakness and elevated CPK. Additional uncommon side effects include GI irritation, constipation, skin rash, allergic reactions, or hematologic reactions, headache, upper respiratory infection, renal failure and tendon rupture.

L-arginine is an amino acid normally present in the diet. It is not a prescription item, and is sold OTC. Most adverse effects occur only with parenteral injection of L-Arginine in large doses. Adverse effects are uncommon, and can include GI symptoms, including stomach discomfort, diarrhea, nausea, vomiting, bloating and abdominal cramps; renal toxicity, including elevated BUN and creatinine levels; cutaneous flushing; hypotension; endocrine effects

including release of growth hormone, insulin, glucagon and prolactin; numbness and headache.

Sapropterin (BH4; Kuvan) is a synthetic preparation of the dihydrochloride salt of naturally occurring tetrahydrobiopterin (6R-BH4 or BH4). It can produce gastrointestinal side effects, including nausea (4%), abdominal pain (5%), diarrhea (8%) and vomiting (8%); headache (15%); and upper respiratory symptoms –nasal discharge (11%), pain in throat (10%), and upper respiratory infection (12-17%). Gastritis, spinal cord injury, streptococcal infection, testicular cancer and urinary tract infection have occurred with Kuvan treatment, but were considered not caused by BH4.

No specific information is available on the conjoint risks of use of the three drugs in this study.

Other risks are claustrophobia when in the MRI scanner; and the possibility of further renal toxicity from gadolinium administration in patients who have pre-administration renal insufficiency (who are excluded). Adverse effects related to gadolinium contrast injection, which occur in less than 1% of patients without renal disease, can include transient headache, nausea,and taste perversion; and extremely rarely, an anaphylactic reaction. Subjects with claustrophobia may be given 0.5 – 1.0 mg Klonopin orally. This may cause drowsiness, slowing of heart rate, or rarely an allergic reaction.

Blood Draw

Blood samples will be taken by inserting a needle into a vein in the arm and withdrawing a sample of blood. It is fairly common for a harmless “black and blue” mark to appear at the site of the vein puncture. Occasionally, patients become dizzy as a consequence of having blood drawn and rarely someone may faint; infection at the puncture site is a rare complication..

Electrocardiogram

The risks include skin irritation and a rash from wearing or removing the patches that stick to the subject’s skin or from the gel that is used with them.

Loss of Confidentiality

The patient’s personal information could be lost or exposed. This is very unlikely; the study personnel will do everything possible to assure that the information is protected.

Questions regarding cardiac, respiratory, upper and lower GI, urinary symptoms, joint or muscle pains will be sought from the patient and caregiver at every visit. Complete blood count, metabolic profile and urinalysis will be obtained each month. The electrocardiogram procedure has no risk for the subject. Any change in heart rate, rhythm, or cardiac integrity occurring during the study will be evaluated at the end of the study by an electrocardiogram.

The hepatotoxicity of simvastatin will be minimized by obtaining baseline liver function tests (LFT's) , and excluding subjects with abnormal liver function (AST or ALT 2x normal). In addition, LFTs will be obtained every month prior to starting the next cumulative drug treatment. CPK levels will be obtained monthly.

Subjects who experience claustrophobia will be prepared for the scanning experience with Klonopin, 0.5 mg orally one hour before the study; repeated one half hour before the study if necessary. Patients with renal insufficiency will be excluded from participation in the study to avoid potential renal toxicity with gadolinium contrast medium.

If subjects or their caregivers withdraw from the study for any reason, they will be asked to return as above. If the subject is unable to tolerate one of the three drugs, they may continue in the study with those drugs that are tolerated, with the collection of data as if they had remained fully involved.

16) **Potential Benefits to Subjects***

AD is a progressive dementing disorder, regarded as one of the most catastrophic conditions that can affect elderly individuals. At present there are no treatments that have been shown to alter the course of the condition, although several drugs can ameliorate the severity of the dementia to a moderate degree. The present study regimen is intended to modify the presumptive underlying cause of the AD- i.e., impaired microvascular function. The treatment is given for 4 months; it is not known whether this modulation of cerebral microvascular endothelial function can slow the progress of the disease, or for what period any beneficial effect may persist. The opportunity to slow, reduce or arrest the progression of AD, however, could be of significant benefit to subjects.

17) **Vulnerable Populations***

All subjects included in this study are assumed to have some degree of cognitive impairment, since they have mild or moderate Alzheimer's Disease. As a result, all subjects are required to have a responsible caregiver give consent to the study, in addition to the subject her/himself. In addition, the caregiver must have daily contact with the experimental subject, and will have telephone access to the PI to report any adverse events, whether related to the drugs involved, or otherwise not involved. Subjects will be particularly closely monitored because of their cognitive impairment, and will be withdrawn if they appear unduly distressed.

Risks to subjects are considered to be minimal since the drugs being used are well-known, FDA approved, and have minimal risks. Subjects, and their responsible caregivers give assent for the study.

18) **Multi-Site Research***

N/A

19) **Community-Based Participatory Research***

N/A

20) **Sharing of Results with Subjects***

Study results are available to be shared with the subjects and/or responsible caregivers after the conclusion of their participation (4 months), when the data have been analyzed. Those subjects, or responsible family members, are informed that they may inquire regarding changes in cognitive function and cerebral blood flow. They are also notified during the course of the study of any abnormal findings on their testing following each visit.

The subjects' primary care physicians are kept informed regarding any abnormal laboratory or clinical findings; any adverse events; and any improvement in the subjects' performance or cerebral blood flow. The information is conveyed immediately, in the case of an adverse event, by e-mail, telephone, or (for internal physicians at UMass) via the electronic medical record system. Communication with the primary care physicians is maintained as needed throughout the 4 months of the study.

As noted, since this is an unblinded pilot study, routine laboratory data are recorded in the Allscripts electronic medical record to be available to the subjects' primary care physicians.

21) **Setting**

All studies described are carried out in the facilities of the University of Massachusetts Memorial Medical Center and Medical School. Clinical examinations and psychometric testing are done in the Neurology Clinic on Level A of UMass Memorial Medical Center, University Campus. Imaging studies are carried out in the University of Massachusetts Medical School Advanced MRI facility in the A-level of the medical school, using the 3-Tesla Siemens MRI scanner.

As noted above, subjects are recruited from any of 6 sites: UMass Neurology Clinic patients seen for early dementia and/or their legally-responsible caregivers, may be asked if they would be interested in participating in the study; an advertisement (see attached) will be sent to Daycare centers and Senior centers in the Worcester vicinity; the Massachusetts/New Hampshire Alzheimer Association has been notified of the availability of the study, and asked to publicize it; word of mouth among patients in the Neurology Clinic at UMass and affiliated hospitals, and physicians will continue to be used to publicize the availability of the study; newspaper advertisements in local newspapers in the Worcester vicinity will indicate the availability of the study; the trial is listed in clinicaltrials.gov, and family members or patients can respond to that listing.

The study is carried out entirely at UMass Memorial Medical Center and Medical School, and follows the regulatory rules and conditions of UMass; no other outside organizations are involved.

22) **Resources Available**

Principal Investigator

The PI is a resident physician training in neurology and psychiatry at UMASS. She will devote approximately 20% time to this study. Each subject's visit requires approximately 4 hours of face-to-face time with the subject and family. Additional time is devoted to scheduling; data collection, recording and analysis; communication with subjects and family members; writing abstracts and manuscripts; and coordination of the study. The PI is responsible for all aspects of the study, while under supervision of the faculty advisor.

The change of the PI was informed to all current and former study participants via phone calls by the PI herself.

Faculty Advisor

The faculty advisor will be Dr. Nils Henninger, M.D. Dr. Henninger is an Assistant Professor of Neurology and Psychiatry at UMASS. He is a vascular neurologist who is well published with a strong research interest in traumatic brain injury and ischemic stroke. He is available for clinical concerns, data analysis, abstract and manuscript writing and editing. He is responsible for overseeing the progress of the study in a supervisory role.

Staff

Clinical Trials Coordinator. Arranging visits; obtaining drug; managing specimens; maintain documents; adverse event monitoring and reporting; IRB communications and continuing review, communication with subjects, performance of the MMSE, and other psychometric tests or research procedures as trained.

Sub-Investigators: sub-investigators will include neurologists, imaging specialists, neuropsychologists and radiologists. Each sub-investigator will be a MD, DO or PhD. Experience and specific training in conducting neuropsychological testing; patient examination; assisting with arrangements for MRI scanning; consent subjects; take medical history; conduct physical examination. Involvement in writing, presentation of data. May be in residency/fellowship training, or faculty level. Carrying out arterial spin labeling and perfusion imaging studies in MRI setting. Analyze imaging data to determine global and focal cerebral blood flow. Collaborate with imaging, cerebral blood flow studies.

In addition, we will share coded, anonymous data with external collaborators. We will advise collaborators to obtain any approvals required by their home institution prior to analyzing the data. We will

never break the code for these individuals or try to re-identify subjects, and the use is limited to this research project. Data will be transmitted by DVD (password protected when possible) or by secure email or by a UMMS IT approved means.

Facilities available

The University of Massachusetts-Memorial Medical Center is available for any medical problems that may arise in the course of the study. The UMass Memorial hematology and chemistry laboratories are involved in the processing of specimens for routine following of subjects.

Communication.

Personnel involved in the study are individually informed regarding the subject involvement and scheduling for procedures. Meetings of the involved staff are conducted at 3-6 month intervals as necessary to inform all members of updates, subjects, responsibilities, data.

23) Prior Approvals

All personnel involved with the MRI-based scanning must be trained/retrained annually in safety measures related to the use of the MRI scanning machine.

All personnel must be trained with, and pass, the CITI website program for clinical trials investigators; retraining is biennial.

IBC approval is not needed for this study because the study only deals with routine blood labs processed in the UMMHC clinical lab.

24) Recruitment Methods

Subjects with AD comprise approximately 10% of all individuals over the age of 65, with the frequency increasing exponentially with greater age. Recruitment, however, requires that the subjects are interested in being involved in a clinical trial; do not have any exclusionary illnesses, and are not currently taking a statin drug. Many patients with AD are seen annually in the UMass Neurology Clinic, and may be eligible for inclusion if they meet criteria and are interested. Other individuals contact the investigator as a result of word-of-mouth, online awareness of the study from the “clinicaltrials.gov” website, the MA/NH Alzheimer Association, and physicians seeking trials for their patients with AD.

Subjects are screened by a clinical evaluation done by the PI. When necessary to ascertain the diagnosis, usual diagnostic tests (standard of care) including a PET scan, a lumbar puncture with analysis of spinal fluid

for beta amyloid and phospho-tau protein, detailed psychometric evaluation or PET scan with Florbetapir for presence of beta amyloid may be used to assure accurate diagnosis prior to offering entry into the study. Medical records provided by other physicians or neurologists who have evaluated the subject for AD may be requested under HIPAA rules.

Telephone interviews may be used for preliminary screening to ascertain any inclusionary or exclusionary conditions.

Methods of recruitment are described in (7) above. No payment is given to subjects.

25) **Local Number of Subjects**

Ten subjects are planned to be included in this pilot study. All subjects are local.

26) **Confidentiality**

This is described in (11, 12 and 13) above. Data are kept in a locked cabinet in a locked room. Only the investigators have access to all the data.

27) **Provisions to Protect the Privacy Interests of Subjects (HIPAA)**

Subjects are evaluated and have contact only with the included investigators, except for (1) scheduling of visits; and 2) drawing of blood in the laboratory by phlebotomists.

Subjects will be evaluated and have contact exclusively with trained members of the research team/investigators, who have been sensitized to the issue of Alzheimer's disease. They are assured of the confidentiality and security of the information obtained.

HIPAA releases are obtained from the subjects and their responsible caregivers. Subjects and caregivers sign releases for information obtained from other physicians who care for them, or who have relevant records regarding their care.

28) **Compensation for Research-Related Injury**

The University of Massachusetts does not provide funds for the treatment of research-related injury. If the subject is injured as a result of participation in the study, treatment will be provided. The subject or his/her insurance carrier will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages is available.

29) **Economic Burden to Subjects**

The subjects are not responsible for any expenses related to this study other than personal travel. Subjects entered in this study will have insurance-related payments to ascertain their baseline neurological and medical status, as would every patient seen in the Dementia and Cognitive disorders clinic at UMass. Once the subjects have undergone the baseline medical/neurological evaluation, and have been accepted into the study, however, all additional expenses related to the study will be paid for by the study funding. If an unrelated medical problem should develop during the course of the study, this will be paid for by the patient's own insurance.

30) **Consent Process**

Consent will be obtained both verbally and in writing. The consent process is carried out with the subject and caregiver, together with the investigator, in an examining room setting in the Neurology clinic. Subjects and caregivers will be provided a written description of the study, including the number of visits, laboratory tests to be obtained, time involved, and possible risks and benefits. This will be discussed with the potential subjects in the presence of the responsible family member/caregiver. They will be given unlimited time to consider their decision to be enrolled or not, either while in the Clinic, or after considering the trial at home; all questions are answered. They will be informed (if patients at UMMC) that no change in care for their general or neurological problems will occur whether they decide to enroll in the study or not. Although some of the subjects will not be totally capable of comprehension of all aspects of the study, the extent of their understanding will be evaluated.

All subjects undergo clinical neurological evaluation, including detailed cognitive evaluation, and psychometric testing as described. This provides the information needed to assess the capacity of subjects to assent to the trial.

In addition, subjects whose cognitive ability is marginally impaired (e.g., MMSE scores of 15 -26) will be recruited. They should all be able to provide some degree of assent; but as noted, in all cases assent will also be required from the subject's responsible caregiver as well.

No non-English-speaking subjects will be included in the study because of difficulties in evaluating their cognitive performance on standard English tests, No children, adolescents or pregnant women will be enrolled in the study.

For the relation to cognitively-impaired adults, see (17)

Dr. DeGrush will notify existing subjects of the ongoing collection of research data during the time period that each subject is on the study drug combination, and for up to 1 year post discontinuation of the study drug.

will happen via a letter to subjects with a HIPAA authorization (no signature required). Subjects will be invited to contact the PI with any questions and many are still in clinical follow-up and will be notified in person as well.

31) **Process to Document Consent in Writing**

Formal consent will be obtained both verbally and in writing from the subjects and from their responsible caregiver. If capable, the subject will sign and personally date the written informed consent.

32) **Drugs or Devices**

The three drugs used in this study are stored in the research pharmacy in the ACC building at UMass. They will be obtained for appropriate visits from the research pharmacists.

i. The drugs are lawfully marketed in the United States.

ii. The research is not intended to be reported to the FDA in support of a new indication for the drugs, nor to support a significant change in labeling of the drugs.

iii. The research is not intended to support a significant change in advertising of the products.

iv. The research does not involve a route of administration or dosage level or use of a patient population or other factor that significantly increases the risks (of decreases the acceptability of the risks) associated with the use of the drug products.

v. The research is conducted in compliance with the marketing limitations described in 21 CFR 312.7.

INVESTIGATOR STUDY PLAN - REQUIRED

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	*X*	*X*	
21 CFR 54	*X*	*X*	
21 CFR 210	*X*		
21 CFR 211	*X*		
21 CFR 312	*X*		
21 CFR 812		*X*	*X*
21 CFR 820		*X*	