Network-Level Mechanisms for Preclinical Alzheimer's Disease Development

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Title	**Network-Level Mechanisms for Preclinical Alzheimer's Disease Development**
Principal Investigator	Shi-Jiang Li, PhD
Study Site	Medical College of Wisconsin
Clinical Trial Phase	Phase II
Study Disease	Alzheimer's disease
Main Eligibility Criteria	• Fluent in English • At least 8 years of education • Healthy APOE 4 carriers and noncarriers • Age 55–75 years • Normal cognitive function • Free of neurological or psychiatric comorbidities
Study Rationale	Levetiracetam (LEV) is a generic drug used in long-term epilepsy treatment, with known pharmacokinetic and pharmacodynamic properties. It is relatively safe and has an acceptable side-effect profile. A novel extended release formulation of AGB101 (220 mg of levetiracetam) was developed to allow for once-daily dosing in amnestic mild cognitive impairment (aMCI). So far, no studies have been conducted to see if a low dose of AGB101can effectively reduce the network dysfunction in cognitively normal subjects who are APOE 4 carriers. The pre-dementia state among APOE 4 carriers can be identified by measuring abnormal brain connectivity with functional MRI. Considering this, use of AGB101 to reduce the network dysfunction in cognitively normal subjects who are APOE 4 carriers should be explored.

Primary Objectives	To determine whether normalization of resting-state connectivity can be achieved in APOE 4 carriers with 2 weeks AGB101 (220 mg, QD) treatment compared with APOE 4 noncarriers receiving the same treatment
Secondary Objectives	To measure cognitive function in APOE 4 carriers and noncarriers at the beginning and end of treatment with placebo

Endpoints	• Primary outcome measurements: Network changes in the bilateral sensory motor cortex regions of the hippocampal functional connectivity after AGB101perturbation (time frame: 2 weeks) • Secondary outcome measurements: Episodic memory performance and neuropsychological test battery as assessed at baseline (Visit 1) and at the post-perturbation procedures (time frame: 2 weeks)
Study Design	This is a randomized, double-blind, placebo-controlled crossover study in which AGB101 (220 mg, QD) or placebo will be administered to 50 healthy 55–75-year-old subjects (comprising 25 APOE 4 carriers and 25 noncarriers), with the order of treatments counterbalanced in a within-subject crossover design. The study consists of prescreening (Visit −1), baseline/screening (Visit 1), a 2-week treatment period (Visit 2), a 4-week washout period (Visit 3), and a 2-week treatment period (Visit 4).
Study Agent/ Intervention Description	AGB101 Levetiracetam Extended Release 220 mg and placebo will be administered orally, QD
Number of Subjects	50, of which 25 are APOE 4 carriers and 25 are noncarriers
Subject Participation Duration	8 weeks: 2 weeks of AGB101/placebo, followed by a 4-week washout, followed by 2 weeks of AGB101/placebo
Duration of Follow up	None
Estimated Time to Complete Enrollment	18 months
Statistical Methodology	We will employ the multivariate regression model to determine the correlational changes between the hippocampal functional connectivity and the episodic memory due to AGB101perturbation; we will assume age, years of education, gender, and gray matter volume as time-invariant covariates.
Safety Assessments	Regular data safety monitoring board meetings will be scheduled to monitor progression of the study and review drug adverse events
Efficacy Assessments	Statistically significant changes between the hippocampal functional connectivity and episodic memory relationship at baseline and at the end of the trial
Unique Aspects of this Study	This is the first study to evaluate the safety and efficacy of AGB101 to regulate brain connectivity in cognitively normal subjects at risk for dementia.

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1. STUDY OBJECTIVES

Neuropathological, structural, and functional changes related to Alzheimer's disease (AD) may begin insidiously, 20 to 30 years before the manifestation of clinical symptoms. This long, preclinical phase of AD provides a critical window of opportunity to conduct early disease intervention. In this study, we will **test our hypothesis that,** ***during the preclinical AD developmental phase in cognitively normal (CN) older subjects with the apolipoprotein E ε4 (APOE 4) allele, decreased abnormal hyperfunctional connectivity can be correlated with improved episodic memory (EM) using a perturbation, such as AGB101 low dose levetiracetam extended release formulation*** The major pieces of foundational evidence that support our hypothesis are described below.

1.1. Justification of Selecting CN Subjects with the APOE 4 Allele

It is well recognized that age and the APOE 4 allele are the most important risk factors for late-onset AD. It is known that APOE 4 allele carriers have a higher risk of converting from CN to mild cognitive impairment (MCI) than noncarriers (1-3). In fact, more than 50% of AD patients are carriers of the APOE 4 allele (4-6). Converging evidence shows that CN, older individuals with the APOE 4 allele manifest functional network disruption (7-13), increased Aβ accumulation (14), decreased memory function (7, 8, 12), and decreased brain volume (15-18), consistent with AD patterns. It is likely that these patterns are detectable at the preclinical AD stage. If we are able to elucidate the mechanisms linking preclinical network disruption and the emergence of clinical symptoms, we can use this critical window of time for potential intervention with disease-modifying therapy. It is evident that selecting CN subjects with the APOE 4 allele will facilitate such a preventive strategy.

1.2. Objective

A low dose of LEV (125 mg, twice daily [BID]) can reduce task-induced hippocampal hyperactivity and improve fMRI task performance in human subjects with MCI. Pioneering studies have demonstrated that, among several antiepileptic drugs, LEV suppresses neural hyperactivity and reverses synaptic and cognitive deficits in AD mice models (19-21). Recent research further demonstrates that a low dose of LEV can reduce task-induced hippocampal hyperactivity in human MCI subjects (22, 23). This proof-of-principle human study suggests that abnormal neural activity may deteriorate memory function (24) and that AD-related neural network dysfunctions are an early and potentially treatable disease component (25-31). So far, no data are available to demonstrate if LEV perturbation can reduce abnormal network activity and concurrently improve memory decline in **CN, older subjects with the APOE 4 allele.** To fill this gap, our specific aim follows:

Specific Aim 1. Determine network-level changes in hippocampal functional connectivity (HFC) after a low-dose AGB101 levetiracetam extended release perturbation (220 mg, QD) for a 2-week period in a randomized, double-blind, and placebo-controlled study with 50 cognitively normal (CN) older subjects (ranging in age from 55 to 75 years), of which 25 are APOE 4 carriers and 25 are noncarriers, and correlate the network changes with the EM before and after a 2-week perturbation with a low dose of AGB101 or placebo.

1.3. Outcome Measurements

The following outcome measurements will be used to test our proposed hypothesis:

- **Primary outcome measures:** Network changes in the bilateral sensory motor cortex regions of the HFC after AGB101 perturbation (time frame: 2 weeks)
- **Secondary outcome measures:** EM performance and neuropsychological test battery as assessed at baseline and the post-perturbation procedures (time frame: 2 weeks)

1.4. Impact of the Proposed Study

Currently, no FDA-approved therapy exists for CN, older subjects who have abnormal functional connectivity and an increased risk for progression to dementia. If this mechanistic approach is successful, we will lay a conceptual framework for future clinical preventive trials in CN subjects with abnormal functional connectivity. Analogous to the use of baby aspirin to prevent the risk of cerebrovascular stroke, we hope a daily low dose of AGB101 will effectively prevent or slow memory decline.

2. BACKGROUND AND SIGNIFICANCE

2.1. Background

Substantial evidence supports our network dysfunction hypothesis that pathophysiological events may be "upstream" of amyloid-β_{1-42} protein (Aβ) accumulation: **1)** Early network dysfunction has been reported before detectable Aβ formation (9) in certain subjects, such as young APOE 4 carriers (10, 11, 32), supporting the antagonistic pleiotropic trajectory and insidious network dysfunction in Alzheimer's disease (AD) progression (33). **2)** Abnormal hyperactivity stimulates Aβ formation and toxicity (26-31, 34), as evidenced by the fact that the Aβ-binding regions overlap the default mode network (DMN) regions(12, 13, 35-37). **3)** Subjects with AD risk factors, including APOE 4 carriers (13, 38) and individuals with amnestic mild cognitive impairment (aMCI) (39-41), manifest network dysfunction abnormalities. **4)** Network dysfunctions have been found in the DMN, salience network, executive control network, dorsal attention network, etc. (12, 42), consistent with AD pathology. **5)** Abnormal network activity impairs memory function (24), and different dementia types target specific neural network sets (43). **6)** Most importantly, recent pioneering studies have demonstrated that antiepileptic drugs, such as LEV, target network dysfunctions by suppressing neural hyperactivity and reversing synaptic and cognitive deficits in AD rodent models (19-21) and in aMCI subjects (22). Both hyperactive (44) and hypoactive (45) circuits can have a negative impact on the disease process. In the present study, we will robustly identify and measure the dysfunctional networks as biomarkers in CN older subjects.

Abnormal hyper-HFC at the network level may be upstream of the underlying neurodegenerative processes. Recently, we published an article that discusses use of the rigorous event-based probability model to comprehensively demonstrate that, during the decade-long progression from the preclinical phase of AD to overt AD dementia, certain neuropathological, structural, and functional events (measured by various biomarkers) occur sequentially. Abnormal functional connectivity occurs first, followed by a decrease in the level of Aβ and an increase in the level of phosphorylated tau proteins in the cerebrospinal fluid; in turn, there is a decrease in structural brain volume, MCI, and the full manifestation of AD dementia. These results indicate that abnormal hippocampus hyperfunctional connectivity at the network level could be upstream of the underlying neurodegenerative processes, even before the level of Aβ is detectable in the brain (46, 47).

2.2. Significance

If this mechanistic study succeeds, we will be able to **1)** provide conceptual proof that AD development can be prevented or delayed by targeting dysfunctional neural networks at the preclinical phase, thus facilitating early intervention, even in CN, older subjects (48); **2)** identify subject groups that have no clinical symptoms but have hyperfunctional connectivity (e.g., APOE 4 allele carriers who are at high risk for AD development), which may prove to be a powerful strategy to decrease or halt cognitive decline; **3)** monitor whether drug intervention reaches specific neural network targets and assess the network level responses; **4)** predict if the network activity modulation can concurrently improve memory decline; and **5)** provide essential information needed to prepare future clinical preventive trials. The long preclinical phase provides a critical window of time for potential intervention with disease-modifying therapy.

This study provides network-level mechanisms that emphasize that dysfunctional networks at the preclinical phase can be targeted for secondary prevention at the asymptomatic stage before significant cognitive impairment. Specifically, we will focus on the following:

- **Preclinical AD Development Phase.** Currently, three clinical trials are being conducted with LEV in the AD research field. Two are testing whether LEV can help AD subjects control seizure-like activity and provide cognitive benefits to AD patients, particularly in individuals with silent epileptic activity. The other is testing whether the daily use of low-dose LEV (125 mg, BID) administered to MCI subjects can improve memory function. Details of these three trials are presented on the clintrials.gov website (https://clinicaltrials.gov/ct2/show/NCT01554683, https://clinicaltrials.gov/ct2/show/NCT02002819, and https://clinicaltrials.gov/ct2/show/NCT01044758, respectively). In contrast, we propose studying **CN subjects with the APOE 4 allele** to reveal if the reduced hyper-HFC and improved EM decline are correlated. If this study is successful with CN subjects, we can take full advantage of this critical window of time (i.e., the long preclinical phase before significant cognitive impairment) and potentially intervene with disease-modifying therapy.

- **Enriched Testing of Subjects with APOE 4 Allele.** As stated, more than 50% of AD patients are carriers of the APOE 4 allele (4-6). The APOE 4 allele is confirmed as the most important genetic risk factor for late-onset AD. The APOE polymorphism modulates the risk of late-onset AD in an isoform-dependent manner (ε4>ε3>ε2) (49, 50). The APOE 4 allele accentuates cerebral amyloid deposition, structural and functional abnormalities, and memory decline in cognitively healthy adults. By treating APOE 4 allele carriers at high risk for AD development with a daily low dose of AGB101 (220 mg, QD) at the preclinical phase, it will be possible to determine if hyper-HFC is reduced and the memory decline is improved.

3. RATIONALE

3.1. General Information Regarding LEV

LEV (Keppra®) is FDA approved worldwide. It is the most commonly approved drug for adjunctive treatment of partial, primary generalized tonic-clonic, and myoclonic seizures for use in adults and children as young as one month. LEV has a novel structure and unique mechanisms of action, although detailed mechanisms for its antiseizure activity are still unclear (51). Unlike other antiepileptic drugs, LEV's action mechanisms appear to involve neuronal binding to synaptic vesicle glycoprotein SV2A (52), inhibiting calcium release from intraneuronal stores, opposing the activity of negative modulators of GABA- and glycine-gated currents, and inhibiting excessive synchronized activity between neurons (53). In addition, LEV inhibits N-type calcium channels. This is believed to impede impulse conduction across synapses (54). LEV is associated with rapid and complete absorption, high oral bioavailability, minimal metabolism, and primarily renal elimination. It lacks cytochrome P450 isoenzyme-inducing potential and is not associated with clinically significant pharmacokinetic interactions with other drugs, including other antiepileptic drugs (55). AGB101 has been developed as a novel extended release formulation of low dose levetiracetam (below clinically marketed doses for epilepsy) for slowing the progression of amnestic mild cognitive impairment. AGB101 is formulated at a strength of 220 mg consisting of Levetiracetam, Hypromellose (Methocel K15M Premium CR), Colloidal Silicon Dioxide, Silicified Microcrystalline Cellulose (ProSolv HD90), Magnesium Stearate and coated with Opadry.

3.2. Justification of Treatment Selection

So far, no studies have been conducted to see if a low dose of LEV can effectively reduce the network dysfunction in CN subjects who are APOE 4 carriers. As described above, LEV is a generic drug used in long-term epilepsy treatment, with known pharmacokinetic and pharmacodynamic properties. It is relatively safe and has an acceptable side-effect profile. In addition, we have seven specific and fundamental reasons to select LEV as the perturbation in this study. **1)** Pioneering research has tested the concept that excess activity in the hippocampus is a dysfunction condition that may be key in underlying, age-related impairment in hippocampal-dependent memory processes. With a rat model, Koh et al. showed that a low dose of LEV can improve memory in aged rats (19). **2)** A comprehensive study showed that, with the human APP mice model, acute LEV treatment reduces abnormal activity spikes. Chronic LEV improves learning and memory, and reverses behavioral abnormalities and synaptic deficits in the hippocampus (20). With a transgenic mouse model of AD (5XFAD mice), Devi et al. recently suggested that suppressing overexcitation with acute LEV treatment around the time of acquisition or early consolidation of memory may be sufficient to reverse memory decline associated with aging (21). **3)** A recent human study showed that LEV treatment (125 mg, BID) for two weeks suppressed task-induced hyper-blood oxygen level dependent (BOLD) signals in aMCI subjects (22). **4)** LEV has minimal interactions with other drugs. This is significant because older subjects often take many other drugs. **5)** Most importantly, because LEV may impede impulse conduction across synapses and suppress neural hyperactivity (23), we can employ the resting-state functional magnetic resonance imaging (rfMRI) method to detect and determine how LEV modulates or normalizes dysfunctional networks, and whether the normalization of network functions can stop or delay EM decline or even improve memory. **6)** The selection of low LEV doses. Within a low dose range between 5–10 mg/kg in a rat model, LEV effectively suppressed hyperactivity and improved memory performance (19-21, 55). Further findings demonstrated that a low dose of LEV improves memory in aged rats with cognitive impairment, but it does not at high doses (19). Many studies showed that LEV treatment at high doses creates a loss of antiepileptic efficacy during sustained treatment (20, 56-58) in rodents and in a small portion of patients with epilepsy (59, 60). For the present study, we have selected a 220 mg, QD dose of AGB101 levetiracetam extended release which is about 10 times less than the clinical antiepileptic dose, for CN subjects carrying the APOE 4 allele. **7)** Selection of duration of LEV perturbation. According to an animal model (20), although acute LEV can effectively suppress hyperactivity, the transient reduction of the abnormal spikes did not reverse abnormal behaviors until the chronic LEV

reversed the abnormalities in synaptic activity-related proteins. This result suggests that long-term LEV perturbation is necessary to provide the time for network adaptation and normality. As such, we designed a 2-week, low-dose LEV perturbation study to address two questions: **One**, can a low dose of LEV decrease abnormal hyper-HFC? **Two**, will the decreased functional connectivity ameliorate EM decline?

3.3. Preliminary Results

Higher HFC activity correlates with greater EM decline in CN APOE 4 carriers. To investigate if high HFC activity is detrimental to EM in CN APOE 4 carriers, 21 CN subjects with APOE 4 (aged 66.62 ± 5.84 years) and 27 CN subjects with APOE ε3ε3 (APOE 3) (aged 70.11 ± 5.23 years) were recruited in a two-year longitudinal study. Structural and rfMRI data were obtained, and changes in EM scores were recorded. The EM scores included the auditory verbal learning test (AVLT) and logical memory test (LMT), which are the two most common EM assessments and are used in various multicenter studies. These tests also can detect accelerated EM decline in cognitively normal APOE 4 carriers (61). As shown in Fig. 1a, CN APOE ε3ε3 subjects exhibited neural correlates of EM function in the Papez circuit regions. However, the baseline HFC activity does not correlate with the rate of EM decline (data not shown).

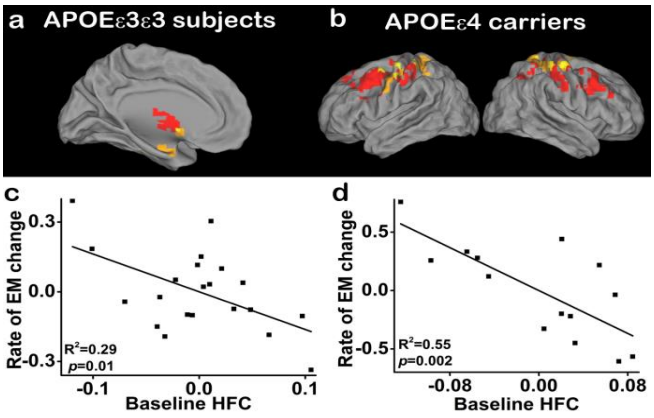


Fig. 1. APOE polymorphisms and their mediation for EM functions. a) Neural correlates of EM functions in APOE ε3ε3 subjects in the Papez circuit region. b) Neural correlates of EM functions in APOE 4 carriers in the bilateral sensory motor cortex regions. c) Associations between baseline HFC connectivity in the bilateral sensory motor cortex and the annual rate of EM decline in APOE 4 carriers are significant. d) The relationship in APOE 4 carriers described in c was validated with an independent dataset from ADNI 2. To calculate the rate of EM change, we converted AVLT and LMT scores to a composite standardized *Z*-score and then divided by the follow-up duration.

Conversely, CN APOE 4 carriers exhibited neural correlates of EM function in the bilateral sensory motor cortex regions (Fig. 1b) and the baseline HFC activity in the sensory motor cortex negatively correlated with the annual rate of EM change (Fig. 1c). These results suggest that APOE ε3ε3 and APOE 4 alleles encode different network systems for EM functions, demonstrating genetic regulation. Further, higher HFC activity predicts a faster annual rate of EM decline in APOE 4 carriers but not in APOE ε3ε3 subjects. The baseline HFC activity in the sensory motor cortex predicts the rate of EM decline: the higher the activity, the faster the rate of decline. Most importantly, these results have been validated with independent datasets from ADNI 2 (Alzheimer's Disease Neuroimaging Initiative) with 14 CN APOE 4 carriers (aged 71.95 ± 4.67 years), as shown in Fig. 1d. These results suggest that activity in Papez circuit commonly mediates EM function without causing EM decline in APOE ε3ε3 subjects, whereas, in APOE 4 carries, the higher activity in sensory motor cortex is detrimental and can be used to predict the annual rate of EM decline (Figs. 1c and 1d). These preliminary results demonstrate the feasibility of detecting abnormal network activity and correlating it with EM decline. We will test our hypothesis that decreased hyperfunctional connectivity can be correlated with improved EM using a daily, low-dose LEV perturbation (AGB101 levetiracetam extended release).

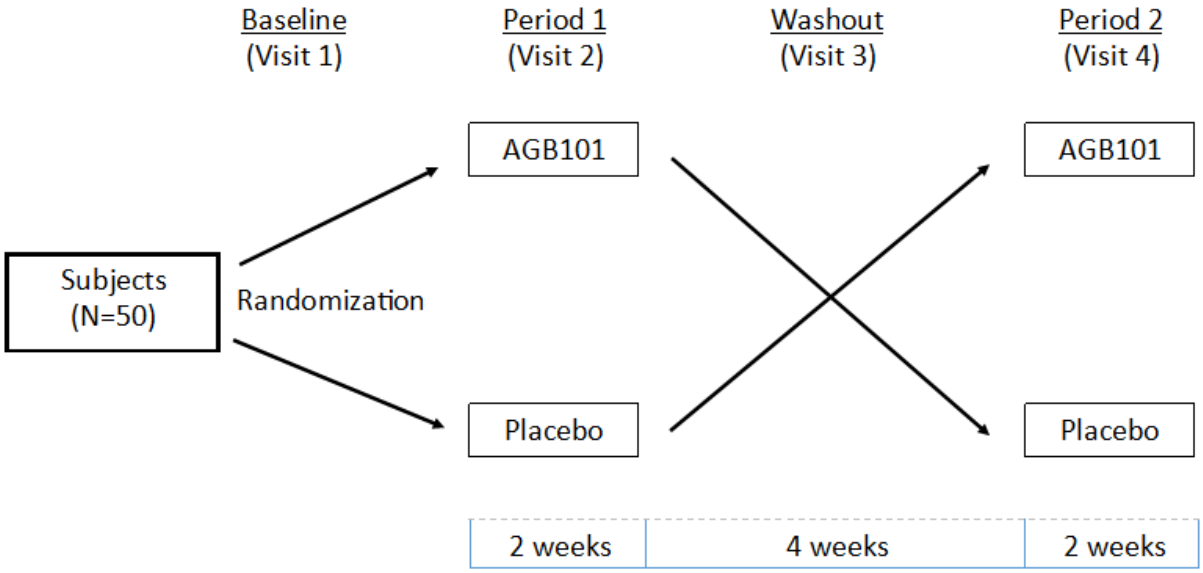
4. STUDY DESIGN

4.1. Overall Design

This study will test the hypothesis that ***decreased abnormal hyperfunctional connectivity can be correlated with improved EM using a daily, low-dose LEV perturbation (AGB101 levetiracetam extended release).*** In this study, there are two study groups: APOE 4 carriers (n=25) and APOE4 noncarriers (n=25). The functional connectivity changes in HFC will be determined in a randomized, double-blind, placebo-controlled crossover manner, in which a low dose of AGB101 levetiracetam extended release (220 mg, QD) or placebo is administered to 50 healthy 55-75-year-old subjects (comprising 25 APOE 4 carriers and 25 noncarriers), with the order of treatments counterbalanced in a within-subject crossover design, as illustrated in

Fig. 2 below. The study procedures are almost identical to those described in Refs (77, 78). In brief, study participants will receive placebo during one treatment period and AGB101 levetiracetam extended release during the other treatment period, with the order of treatments counterbalanced (randomized, double-blind).

Figure 2. Study design and AGB101 treatment protocol



Notes: Of the 50 subjects, 25 are APOE 4 carriers and 25 are noncarriers.
See Table 1 for timing and schedule of procedures.

Visit 1 (baseline) and Visits 2 and 4 (which are separated by a 4-week washout period, -/+ 1 week) include a physical and neurological examination, a neuropsychological assessment, and fMRI scans. At baseline visit and the third visit, each subject will be provided with the study medication. At the end of the period 1, period 2 and washout period, each participant will have a blood draw. Treatment compliance will be assessed by reviewing participant self-reports at the end of each period, bottle return and pill count, and analyzing of AGB101 levetiracetam blood values at each study visit. The study team is blind to the APOE status of the participants and AGB101 levetiracetam blood levels until the final group analysis of the data. The company Agenebio, Inc. will provide AGB101 levetiracetam extended release tablets as well as placebo. The Froedtert Hospital Investigational Drug Service will randomize participants to the treatment conditions, dispense the study medication, and control blinding and unblinding of study data according to standard clinical trial procedures. Data safety is monitored by three data safety monitoring board (DSMB) members who are not related to the study, in collaboration with the Froedtert Hospital Investigational Drug Service.

4.2. Inclusion and Exclusion Criteria

Inclusion Criteria

- Fluent in English
- At least eight years of education
- A Geriatric Depression Scale (GDS) (62) score < 6 and Hachinski Ischemic Score ≤ 4
- Normal general cognitive function as well as 1) normal memory function, documented by MOCA score of 23 or greater, and a RBANS Delayed Memory Index score of 85 or greater.

Exclusion criteria

- Neurological diseases, such as Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, seizure disorder, subdural hematoma, multiple sclerosis, or a history of significant head trauma followed by persistent neurologic deficits or known structural brain abnormalities
- Presence of major psychiatric disease or chronic unstable medical conditions
- History of drug abuse
- History of alcohol abuse (4 or greater drinks per day on average)
- Contraindication to MRI
- Known clinically significant abnormalities in B12 or thyroid function tests
- End Stage Renal Disease (ESRD)
- Hemodialysis (HD)

4.4. Subject Recruitment

We will screen about 150 CN subjects ranging in age from 55–75 years to obtain 25 APOE 4 carriers and 25 noncarriers, according to a 20–25% prevalence of the APOE 4 allele among this population (66). Recruitment efforts will be conducted in the metropolitan Milwaukee, WI, area, which has more than 1 million inhabitants. The Medical College of Wisconsin (MCW) Dementia Research Center has extensive experience in recruiting from the clinic and the Milwaukee community, using flyers, radio announcements, press releases, and community presentations. We also will contact the University of Wisconsin-Madison based Alzheimer Research Center to recruit to potential volunteers registered with that center and residing in the southeastern counties of Wisconsin.

Study Visits and Procedures

5.1. Schedule of Visits

At **screening** (**Visit −1**) informed consent (IFC), blood (or buccal swab) for APOE genotyping, demographics, medical history, MRI safety screening, and MOCA/RBANS will be obtained in one or more visits. After a subject's APOE genotype is known, the subject will then be invited to return for the **baseline visit**. At **baseline** (**Visit 1**), the subject will undergo a physical and neurological exam, and neuropsychological assessments. Neuropsychological assessments will be reviewed by the clinician neurologist as part of the inclusion and exclusion criteria. Those subjects who meet the inclusion criteria and have none of the exclusion criteria will be enrolled in the study. Enrolled subjects will undergo fMRI scans, be randomized to a treatment group, and receive the study medication for Period 1. **Visit 2** will be scheduled on the last day of Period 1; at this visit, a brief medical and neurological examination, a neuropsychological assessment, a blood draw, and fMRI scans will be administered. **Visit 3** will be scheduled at the end of the washout period. At this visit, each subject will have a blood draw and be provided with the study medication for Period 2. **Finally, Visit 4** will be scheduled for repeat cognitive tests, blood draw and fMRI scans as Visits 1 and 2. Treatment compliance will be monitored by participant self-report at the end of each treatment phase, bottle return and pill count, and analysis of AGB101 levetiracetam blood values at each visit during the study protocol.

Refer to Table 1 for a schedule of study procedures.

5.2. Subject Retention and Compensation

The study staff will monitor treatment compliance as well as potential adverse events (AE). The study staff will apply the standard operating procedure currently in place to monitor AEs. Subjects will be compensated for their participation in the study.

5.3. Early Termination Visit

If a subject decides to exit the study, a termination visit will be scheduled. This will include all assessments normally performed at Period 2 visit. In addition, any incidental imaging findings will be dealt with in accordance with the standard operating procedures.

5.4. Study Procedures

5.4.1. Informed Consent

Participants will provide written informed consent twice: first at the screening phase for collection of blood (or buccal swab) for APOE testing as well as for other screening procedures; the second consent is for qualified individuals to participate in the main intervention phase and all related study procedures. The consent process will involve distributing a full consent form to the potential study participant and a thorough presentation on the purpose and risks of the study, and an explanation of study procedures by the staff member obtaining consent. Sufficient time for questions, discussion, and the subject's decision will be allowed for proper informed consent by the participant. Once the participant fully understands the study protocol as outlined in the consent form, he/she will acknowledge his/her consent with a signature that is placed directly below the text on the consent form. Each participant will receive a complete copy of the signed consent form for his/her personal records. The study coordinator will keep the original signed copy for study records.

5.4.2. Blood Sample (or Buccal Swab)

A blood sample (or buccal swab) will be collected at the prescreening period to determine whether the subject is a carrier or noncarrier of the APOE 4 gene.

5.4.3. Demographics, Medical History, and Physical and Neurological Exam

Demographic information, a comprehensive medical history and neurological examination with vital signs occur at the screening visit, and vital signs occurs on the last day of each study period. (see table 1) Data are collected from participants using HCP and National Alzheimer's Coordinating Center (NACC) procedures and forms, including demographic information (age, gender, race, ethnicity), medical and surgical history, hospitalization history, medications and supplements, and family history of Alzheimer's disease. We will also ask questions pertaining to diet, sedentary/active lifestyle, sleep history, fluency in languages, marital status, socioeconomic status, education, and work history, and also obtain information on smoking, alcohol use, caffeine use, and drug use.

5.4.4. Neuropsychological Assessments

AD is characterized by progressive EM deficit at the predementia stage. Current AD diagnosis guidelines recognize EM deficit as a core clinical criterion for typical AD diagnosis (67, 68) and recommend objective decline in EM performance as the starting point of AD clinical onset (69). Practically, AVLT and LMT are the two most common EM assessments that are used in various multicenter studies, including the Alzheimer's Disease Neuroimaging Initiative and the Australian Imaging, Biomarker and Lifestyle Study of Ageing. These tests also can detect accelerated EM decline in cognitively normal APOE 4 carriers (61). Therefore, this study will employ AVLT as objective measurements to evaluate the EM decline and the changes to LEV perturbation. For comprehensive cognitive measurements of CN subjects, all enrolled subjects will be administered the WAIS-IV Digit Span and Coding, RAVLT, Boston Naming Test, and Verbal Fluency: FAS & Animal Fluency.

5.4.5. Neuroimaging Assessments

We will conduct imaging on our research-dedicated GE 3T MRI scanner (Signa Premier) equipped with a 48-channel receive-only head coil, which is FDA-approved, or in situations where a back-up scanner is needed, on our research-dedicated GE MR750 3T MRI scanner with 32-channel receiver coil and software version DV26. We will use adapted multiecho image sequence to conduct this project. Below, we briefly describe the imaging acquisition, parameters, and image types. **The first type of measurement is the T1-weighted high-resolution anatomical images. The second type of measurements is task-driven and resting-state fMRI scans.** The 3T fMRI data will be acquired following the adapted General-electric multiecho image sequence, the acquired multiecho images can be effectively denoised with 3.75x3.75x3.75 mm spatial resolution (70). For fMRI scans, one is for EM and the other is for finger tapping**.** We will utilize a memory task, which is being employed currently for the Alzheimer's Disease Connectome Project in healthy older adults, including participants with memory disorders. The task involves serial presentation of novel (NV) and previously learned (PL) pictures obtained from a published normative set. During the fMRI scan, NV or PL items are presented sequentially for the duration of the scan, and the task is to decide whether the present item is new or previously learned. The task elicits activation in the bilateral ventral and medial temporal lobes, bilateral hippocampal, and fusiform regions. For the Finger tapping task, we will utilize a task developed at MCW. The task follows a block design where participants alternate between finger tapping and rest, in response to on-screen instructions. This task robustly activates the motor network. For the resting-state fMRI scans, two 8-min scans will be acquired with phase up and phase down encoding, respectively. The **third type is the arterial**

spin-labeling (ASL) cerebral blood flow (CBF) perfusion images. *The data acquisition scans, plus patient handling time in and out of scanner, will result in an approximately 1-hour scanning session.*

Prior to fMRI scans, subjects will undergo MRI safety screening and mock scanner training, as detailed in sections 7.3. and 7.4., respectively.

5.4.6. Blood Draws

All subjects will undergo a blood draw at the last day of each treatment period and on the last day of the washout period. Samples will be labeled with the study name, study visit number, contents of each collection vial, and the date of collection. Tubes may also be labeled with a barcode for sample management.

5.4.7. Randomization, Study Drug Dispensation, Administration, and Compliance

The Investigational Drug Service at Froedtert Hospital will randomize participants to the treatment conditions.

In Period 1, all study participants will receive treatment with either AGB101 (220 mg, orally, QD) or placebo; in Period 2, all subjects will crossover and receive the other treatment. This is a double-blind study, so both the participants and study team will be blind to the treatment received.

All study treatments (drug and placebo) are dispensed in identical nondescript tablets by the Investigational Drug Service at Froedtert Hospital. Tablets are provided for daily morning doses. Study drugs will be dispensed at baseline and on the last day of the 4-week (-/+ 1 week) washout period. Subjects will be asked to self-administer treatment QD, once in the morning.

Treatment compliance will be monitored by participant self-report at the end of each treatment phase, bottle return and pill count, and analysis of AGB101 blood values at each visit during the study protocol.

Data Management

6.1. The Froedtert Hospital Investigational Pharmacy system will be used to enroll patients and obtain the randomly assigned treatment group. Data forms will be printed, completed, and entered into a database. The database will be monitored for completeness, consistency, accuracy, and timeliness. No patient-identifying information will be stored in the database. To ensure that data are secure from external violation, computer systems containing study data will be password protected, and physical access to the computer systems will be limited.

6.2. Determination of Changes in Network Activity and Concurrent Improvement in EM. The cognitive and MRI/rfMRI datasets will be obtained at baseline (before placebo/AGB101 administration) and on the last day of each 2-week treatment period. These two time points will be denoted t = 1, 2. The two 25-subject groups, both of which will receive both placebo and AGB101 at different treatment phase, will be denoted g = 1, 2. The set of EM scores for subject *s,* from group *g,* at time *t* will be denoted *Cog*(*s,g,t)*, where s = 1, …, 25; g = 1, 2; and t = 1, 2. The HFC calculated between the hippocampus and the bilateral sensorimotor cotex for subject *s,* from group *g,* at time *t* will be denoted *HFC*(*s,g,t).*

6.3. Functional-Connectivity Changes Due to Perturbation. We employed age, years of education, gender, and gray matter (GM) volume as time-invariant covariates to obtain residual variables to determine the HFC changes due to perturbation. We will model the *HFC* as a function of these nuisance regressors, using the following multiple linear regression equation, and retain the residuals as $\boldsymbol{HFC^{+}_{Res}(s,g,t)}$ for further analysis:

$$\boldsymbol{HFC(s,g,t) = \beta_0 + \beta_1 Age(s) + \beta_2 Edu(s) + \beta_3 GM(s) + \beta_4 CSF(s) + \beta_5 Gender(s) + Res(s,g,t)},$$

where $\widetilde{\boldsymbol{HFC}}^{+}\boldsymbol{(s,g,t)} \equiv \boldsymbol{Res(s,g,t)}$. This regression will be performed using all of the data (i.e., all subjects, all groups, and all times). The GM measurement will be conducted with GM concentration and hippocampal volume measurement, as described in our publication and others (71-74). We will test the *residuals* $\widetilde{\boldsymbol{HFC}}^{+}\boldsymbol{(s,g,t)}$ using *three-factor crossed-nested analysis of variance (ANOVA)*, with fixed factors of *group* and

time and a random factor of *subject* nested within *group* (i.e., all subjects will be tested at all times, but the placebo and AGB101groups will contain different treatment phases). We will test for both group and time main effects, as well as for the group × time interaction. If we find significant main effects or interactions, we will perform post hoc procedures to determine which time and group (i.e., placebo vs. AGB101) combinations are significantly different. In addition to the HFC analysis, we will conduct a DMN analysis 75).

6.4. EM Changes Due to AGB101Perturbation. The two EM scores (AVLT and LMT) will be normalized to *Z*-score by transforming individual raw test scores according to the mean and standard deviation of the scores for all subjects. Then, *Z*-scores of each test will be averaged to obtain individual composite memory *Z*-score, as we have previously published (76). Similar to the above model for HFC changes due to AGB101perturbation, we will model the *Cog* score, using the time-invariant covariates as nuisance regressors:

$$\boldsymbol{\Delta EM(s,g,t) = \beta_0 + \beta_1 Age(s) + \beta_2 Edu(s) + \beta_3 GM(s) + \beta_4 CSF(s) + \beta_5 Gender(s) + Res(s,g,t)}$$

Define $\widehat{\boldsymbol{\Delta EM}}(\boldsymbol{s,g,t}) \equiv \boldsymbol{Res(s,g,t)}$. We will test the residuals $\widehat{\boldsymbol{\Delta EM}}(\boldsymbol{s,g,t})$ using *a three-factor crossed-nested ANOVA*, with fixed factors *group* and *time*, and random factor *subject* nested within *group*. We will test for group and time main effects, as well as the group × time interaction. If we find significant main effects or interactions, we will perform post hoc procedures to determine which time and group combinations are significantly different.

6.5. Prediction of Changes in Correlation Between HFC and EM Before and After AGB101Perturbation. A multiple linear regression is used to model EM as a linear function of HFC and test the difference in slope and intercept between the AGB101and placebo groups. If the subject is in the AGB101group, **I**(**s**,**g**) = 1; if the subject is in the placebo group, **I**(**s**,**g**) = 0.

$$\boldsymbol{EM(s,g) = \beta_{0,0} + \beta_{0,1} I(s,g) + \beta_{1,0} HFC(s) + \beta_{1,1} HFC(s) I(s,g) + Res(s)}$$

Here, **HFC**(**s**) represents baseline HFC for subject *s* (for simplicity, nuisance regressors are not shown).

6.6. Expected Results, Deliverable, and Problems

Through this study, we expect to realize two goals: ***Scientifically,*** we will demonstrate that aberrant HFC represents a primary upstream mechanism that may contribute to cognitive deficits in CN APOE 4 carriers. Further, we will demonstrate that changes in the dysfunctional hippocampal network upon AGB101perturbation can be quantified as a biomarker for measuring network responses. ***Clinically,*** we expect that the short-term changes in network activity after the 2-week perturbation period will lead to a decrease in HFC, and possibly to cognitive improvement. This study can provide conceptual proof for preventive intervention. Using the predictive relationship between HFC and the EM changes, individuals carrying the APOE 4 allele can then be stratified into high and low HFC activity subgroups to further refine the subpopulation. Also, this study will provide information and address the power of adequate enrollment, protocol adherence, subject retention, and safety for future pilot trials.

We may be able to detect HFC changes in the 2-week period after AGB101perturbation as a primary outcome, but we may not be able to detect significant EM changes as the secondary outcome in this short R21 funding mechanism. In a future study, we could extend follow-up to 26 weeks or a year to enable us to detect the slowly declining EM functions.

7.DATA SAFETY MONITORING PLAN

An appointed DSMB will discuss the ethical conduct of the trial, review event definitions and the subject ICF form, and further develop plans for monitoring the data and safety of the trial. The DSMB will meet at least every six months to review the course of the trial and interim data. The DSMB agrees to communicate with institutional review board (IRB)/human subject committees to provide reassurances, as appropriate. There will be three DSMB members with the following areas of expertise:

- Neural mechanism underlining learning and memory in aging and neurological disease
- Aging and memory loss
- Psychology and neuroimaging

7.1. Safety Plan

The safety issues in this study are related to use of LEV and acquisition of fMRI data. This study uses the AGB101 levetiracetam extended release at a dose that is 10 times smaller than the doses typically used to treat epilepsy, the main indication for standard LEV. LEV is associated with rapid and complete absorption, high oral bioavailability, minimal metabolism, and primarily renal elimination. The LEV plasma half-life in adults is 7 ± 1 hour and is unaffected by either dose or repeated administration. LEV is eliminated from the systemic circulation by renal excretion as an unchanged drug, which represents 66% of administered dose. The total body clearance is 0.96 mL/min/kg and the renal clearance is 0.6 mL/min/kg. The mechanism of excretion is glomerular filtration with subsequent partial tubular reabsorption. The metabolite ucb L057 is excreted by glomerular filtration and active tubular secretion with a renal clearance of 4 mL/min/kg. LEV elimination is correlated to creatinine clearance. LEV clearance is reduced in patients with impaired renal function. It lacks cytochrome P450 isoenzyme-inducing potential and is not associated with clinically significant pharmacokinetic interactions with other drugs, including other antiepileptic drugs (55). Among AEs reported over placebo at standard therapeutic doses of 1000–2000 mg daily are pharyngitis (9%), mood swings (4%), dizziness (5%), infection (5%), asthenia (6%), and somnolence (7%). Serious dermatological reactions, including Stevens-Johnson syndrome and toxic epidermal necrolysis, have been reported in at 14–17 days from initiation of treatment.

7.1.1 Adverse Event Reporting

Throughout the course of the study, all AEs will be monitored and recorded on an AE CRF, including the AE's description, start and end date, outcome, seriousness, severity, action taken, and relationship to the study drug. If AEs occur, the first concern is the safety of the study subjects. All AEs will be followed until resolved or stable and the outcome documented on the CRF.

7.1.2 Definitions and Criteria

7.1.3 Adverse Events

Per International Conference on Harmonisation (ICH) E2A: An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Events meeting the definition of an AE **include**:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study
- Signs, symptoms, or the clinical sequelae of a suspected drug interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concomitant medication

Clinically significant abnormal findings (laboratory test results, vital signs, physical examination findings, ECGs, radiologic exams, or other studies) should be recorded as AEs. A "clinically significant" finding is one that affects clinical management, including additional visits, monitoring or referrals, diagnostic tests or alteration of treatment, or that is considered clinically significant by the investigator. A clinically significant finding may be a change in a test that has previously been abnormal but now requires additional action.

When a medical or surgical procedure is performed, the condition that leads to the procedure should be recorded as the AE.

Events that **do not** meet the definition of an AE include:

- Anticipated day-to-day fluctuations or expected progression of pre-existing disease(s) or condition(s) present or detected at the start of the study unless judged by an investigator to be more severe than expected for the subject's underlying condition
- Abnormal laboratory, ECG, or vital sign measurements that are not labelled clinically significant (see definition above)
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Pregnancy
- Overdose in the absence of other AEs will not be reported as an AE in its own right
- Changes in C-SSRS during the course of the study indicating worsening should be evaluated by the investigator for clinical significance, and if clinically significant (eg, alteration in medical care or intervention is required), an associated AE should be recorded, if present. The AE should be the primary underlying clinical manifestation assessed as clinically significant, and not the change in score itself.
- Change in cognitive functioning (improvement or worsening) is also not considered to be an AE.

7.1.4 Serious Adverse Events

An SAE or reaction is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization, with the exception of:
    - Visits to the emergency room or hospital department that do not result in an overnight hospital admission
    - Elective surgery for a pre-existing condition that has not worsened
    - Routine health assessments requiring admission not associated with any deterioration in condition
    - Social admission (lack of housing, family circumstances, etc.)
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (eg, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, development of drug dependency or drug abuse, or malignancy tumors [histologically different from the primary tumor])

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

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. An SAE is not necessarily severe, eg, a hospitalization for a diagnostic procedure must be reported as an SAE even though the occurrence is not medically serious. By the same token, a severe AE is not necessarily serious: nausea of several hours' duration may be rated as severe but may not be considered serious.

7.1.5 Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction (ADR) is a reaction for which the nature or severity is not consistent with the applicable product information (see current Investigator's Brochure). Until product information is amended, expedited reporting is required for additional occurrences of the reaction. Reports that add significant information on specificity or severity of a known, already documented SAE constitute unexpected events. For example, an event more specific or more severe than described in the Investigator's Brochure would be considered "unexpected." Specific examples would be (a) acute renal failure as a labeled ADR with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

Assessing Intensity and Relationship

All AEs will be assessed on 2 descriptive parameters: intensity and relationship to the study drug:

1. Intensity refers to the severity of an event and references the impact on a subject's functioning.
2. Relationship refers to the likelihood that the event being assessed was caused by the study drug.

Intensity

Each AE will be classified according to the following criteria:

Mild:	The AE does not interfere in a significant manner with the subject's normal level of functioning.
Moderate:	The AE produces some impairment of functioning but is not hazardous to the subject's health.
Severe:	The AE produces significant impairment of functioning or incapacitation and is a definite hazard to the subject's health.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the experience should be noted. If the intensity category changes over a number of days, those changes should be recorded separately (with distinct onset dates).

Relationship

Each AE will be assessed as to its relationship to the study drug based on the following criteria. Although the attribution by the investigator will be collected for reported events, for analytic purposes a temporal association with the use of the study drug will be assumed sufficient for at least plausible association.

Not related:	No causal relationship exists between the study drug and the AE, but an obvious alternative cause exists, eg, the subject's underlying medical condition or concomitant therapy.
Related:	There is a reasonable/plausible possibility that the AE may have been caused by the study drug.

When assessing the relationship to the study drug, the following criteria will be considered:

- Temporal relationship
- Positive rechallenge
- Positive dechallenge (resolution upon stopping the study drug, in absence of other intervention or treatment)
- Known class effect
- Biological plausibility
- Lack of alternative explanation—concomitant drug or disease

7.1.6 Reporting Procedures and Requirements

Adverse Events

AE recording will begin after the first dose of study drug is administered the morning after baseline. Thereafter, AEs will be ascertained by asking the subject how he/she has been since the last visit. Any AEs occurring before the start of treatment (ie, before the first dose of the study drug) will be recorded in the medical history only. If the investigator detects an AE in a study subject within 1 month (4 weeks, -/+ 1 week) of the last scheduled follow-up visit this will also be collected. All AEs will be followed until resolved or stable and the outcome documented on the CRF.

Also, the sign, symptom, or disease present before starting the treatment period are only considered AEs if they worsen after starting the treatment period.

The investigator should report all information about AEs on the AE/SAE section of the Case Report Form (CRF). Whenever possible, an AE will be reported using a diagnostic term (eg, “common cold” or “upper respiratory infection” rather than “runny nose, cough, mild fever”) and should be described with the attributes.

Serious Adverse Events

Each AE will be assessed to determine whether it meets seriousness criteria (Section 7.1.2.2). If the AE is considered serious, the investigator should report this event to Agene Biotech as outlined below and to the Institutional Review Board (IRB) & Data Safety Monitoring Board (DSMB) according to their standard operating procedures.

All information about SAEs will be collected and reported by entering the SAE information in the AE CRF. The investigator should send the initial report to the Site PI and to Agene Biotech within 24 hours of becoming aware of the SAE and followed by follow-up reports as soon as possible, whether the events are deemed related to the study drug or not. The information provided in the AE system should be as complete as possible, but minimally it must contain the following:

- Subject number
- Brief description of the SAE (diagnosis or signs/symptoms)
- Serious criteria
- Causality assessment
- Assessment of the intensity of the event

Agene Biotech will receive notification of the initial SAE via an e-mail from MCW Study Team. Site personnel will complete the paper SAE report form, scan and e-mail it within 24 hours to the following address: safety@agenebio.com

SAEs that are ongoing should be followed until resolved or stabilized to a level acceptable to the investigator. Information not available at the time of the initial report (eg, an end date for the AE, laboratory values received after the report, or hospital discharge summary) must be documented on a follow-up form. All follow-up information must be reported in the same timelines as initial information.

Any SAEs considered related to the study drug and discovered by the study team at any interval after the study must also be reported to the Agene Biotech within 24 hours following knowledge of the event.

Sponsor Reporting of Suspected Unexpected Adverse Reactions to Regulatory Authorities (SUSAR)
Per 21 CFR 312.32[c](1)(i)) the Sponsor (MCW) must report in an **IND Safety Report** any suspected adverse reaction to study treatment that is both serious and unexpected. Before submitting an IND safety report, the Sponsor needs to ensure that the event meets all three of the definitions:

- Suspected adverse reaction
- Serious
- Unexpected

The Sponsor/MCW Study Team will submit a **FDA Form 3500A MedWatch** for expedited reporting of all events that qualify for expedited reporting. Refer to the Form FDA 3500A Supplemental Manual for instructions and timelines on mandatory reporting.

Unblinding Treatment Allocation
Generally, only SUSARs for which the treatment allocation of the subject is unblinded should be reported by the sponsor to the pertinent regulatory authorities.

When an event may be a SUSAR, the blind should be broken only for that specific subject, and only if knowledge of the study drug assignment is material to the medical management of the AE. When unblinded to assist in the medical management, the blind for that subject should be maintained for individuals responsible for the ongoing conduct of the study (eg, management, monitors, and investigators) and those responsible for data analysis and interpretation of results at the conclusion of the study (eg, biometrics personnel).

Unblinded information should only be accessible to those who need to be involved in the safety reporting to pertinent regulatory authorities, independent ethics committees/independent review boards (IRBs) and DSMBs, or individuals performing ongoing safety evaluations during the study.

Reporting to Institutional Review Board and Data Safety Monitoring Board
The IRB/DSMB will be notified of any SUSARs according to local regulations and within the designated timeframe. Refer to MCW IRB Reporting Policy. These will be reported to the DSMB in parallel.

7.2. MRI Safety
The MRI scanner device is a U.S. Food and Drug Administration (FDA)-cleared device for safe and noninvasive imaging of the interior of the human body. The GE MR750 MRI uses a 32-channel receive-only head coil, which is an investigational device. See below for further information on the 32-chanel receive-only head coil. The pulse sequences employed in this study were developed based on multiecho sequence by GE Healthcare, which constrains the pulse sequences to operate within the FDA limits for safe operation with respect to gradient switching (dB/dt) and RF power (specific absorption rate [SAR]). The proposed MRI scans are NOT for a use of substantial importance in diagnosing, curing, mitigating, or treating disease or otherwise preventing impairment of human health, and they do not present a potential for serious risk to the health, safety, or welfare of a subject. The scanner also restricts the software from exceeding FDA safety levels. The scanner monitors the SAR for research scans just as it does for all other scans. Thus, the scanner with the software fully engaged operates, from a technical design and functional standpoint, as a **nonsignificant risk** device in accordance with 21 CFR 812. By staying below these limits, the operating conditions of the MRI device are generally deemed, in and of themselves, to make the MRI device a nonsignificant risk device.

The Nova Medical 32-channel head coil (model number NMSC075-32-3GE-MR750) is an investigational device that is not approved by FDA for clinical use. This coil device includes multiple features for safe operation involving human studies. The 32-channel coil is designed and constructed as a receive-only detector of RF signals that are emitted by the brain following the RF excitation generated by the GE Healthcare MRI scanner, which is FDA approved. During the RF excitation by the scanner, the coil device is decoupled (made inactive) through redundant circuitry; thus, the coil device never transmits RF to the subject and, therefore, it has no impact on subject risk or safety.

More specifically, the coil design and construction include the following safety features: **1)** High-voltage breakdown (>2 kV) UL-94V0 flame retardant housing. **2)** Rugged construction to ensure safe operation in case of rough handling. **3)** Active detuning circuitry providing greater than 35 db isolation per element. **4)** High power passive detuning circuits in case primary detuning circuitry fails. **5)** Multiple common mode traps in all receive coil cables. **6)** Minimum of 5 mm spacing between coil conductors and patient contact.

Additionally, the coil was designed and manufactured under an ISO 13485 certified quality management system. As part of this quality system, Nova Medical has conducted a failure means and effects analysis

(FEMA) of this product, and we feel that it is a nonsignificant risk under foreseeable normal conditions when used on the 3T GE X750 MRI scanners at MCW.

The Medical College of Wisconsin has installed a new GE 3T MRI scanner (Signa Premier) equipped with a 48-channel receive-only head coil, which is FDA-approved. We will use the new GE 3T Signa Premier MRI scanner and 48-channel head coil for study subjects. The GE750 MRI scanner with the 32-channel head coil will be used as a back-up for scheduling purposes.

7.3. MRI Safety Screening

MCW-specific magnetic resonance (MR) safety screening procedures will be followed. All participants will be screened for medical devices, implants, and metal prior to undergoing MRI, first at the screening visit (Visit -1) and again prior to entering the scanner room on Visits 1, 2 and 4. If it is necessary to review medical records to confirm contraindication, a review of medical records (e.g., previous surgeries) will take place prior to the scheduled visit. During the screening process, participants will also be questioned about their ability to temporarily remove transdermal patches (such as birth control or nicotine patches). Women of child-bearing potential will be asked to confirm that they are not pregnant when signing the ICF. If a woman has concerns or is uncertain of her pregnancy status, she will be excluded, as the risks of an MRI scan to pregnant women are currently unknown. The risks related to an MRI are minimal for a properly administered visit. The MR technicians are trained and prepared to deal with any problems that may arise.

7.4. Mock Scanner Training

As part of the study, participants will engage in a mock scanner training session. The purpose of this session is to acclimate participants to the scanner and to assess their comfort level, minimize any anxiety, and provide practice and training on all tasks that will be conducted in the real MRI environment. Training in the mock scanner will take approximately 20 minutes and will occur prior to a subject's first imaging session. After the session, any questions or concerns will be discussed. Participants may be excused from the mock scanner training at subsequent study visits.

7.5. Incidental Findings by MRI

The study subjects to be recruited for this study are cognitively healthy. Sometimes, however, a few incidental abnormalities may be found in these study subjects, such as brain tumors, vascular lesions, moderate to severe white matter lesion load, and other neuroradiological abnormalities that would preclude subjects from being included in the analysis, or that are clinical abnormalities requiring follow-up. These subjects will be advised to see their physicians for formal assessment. Dr. Piero Antuono is a board-certified neurologist at MCW. He or a Co-I (MD) will consult with the subjects in such cases.

7.6. Subject Safety

Due to the duration and the low doses of AGB101administered in this study, we anticipate this study to be at minimal risk to the volunteer. We do not expect frequent or severe AEs to be present. Subjects will be instructed to call the study site for intercurrent illnesses. All AEs will be monitored according to the Safety Plan (see Section 7.1). The DSMB board will review the initiation frequency and severity of any AEs. Stopping rules will be invoked for the subject and study if AE are reported above the 5% threshold. Subject safety will be ensured during the study period, and the study may be closed with regular visits to the resolution of the AE. Safeguards to ensure subject confidentiality will consist of maintaining records in a locked cabinet in a locked room in the Department of Neurology research offices. All computers are password protected to further ensure safety.

7.8. Data

AE data analysis will be performed every 2 months. Plans for unexpected problems involving risk to participants or others will consist in notification of the principal investigator in order to activate appropriate medical or professional intervention as needed.

7.9. Efficacy

Evaluation of efficacy will consist of measurement of brain connectivity at the interim point when recruitment has reached 50% of subject with a second fMRI session. Secondary outcome measure of cognitive

assessment will also be reviewed. It is anticipated, however, that with the short, 2-week treatment period, statistical significance may not be reached.

7.10. Feedback Mechanism

Evaluation and response to subjects' complaints will be routinely addressed by the DSMB. The IRB and principal investigator will be notified of significant protocols violations and emergence of unexpected AEs at any time during the study. In addition, a report by the DSMB will be provided to the IRB at the end of the study.

Table 1. Schedule of study procedures.

	Screening[h]	Baseline	Period 1[b]	Washout[c]	Period 2[b,d]
Visit	−1	1	2[e]	3[i]	4[e]
Duration			2 weeks	4 weeks	2 weeks
Informed Consent #1[a]	X				
Blood (or Buccal Swab) for APOE	X				
Demographics	X				
Medical History	X				
Informed Consent #2[a]		X			
Inclusion/Exclusion Criteria		X			
NeuroExam	X				
Vital Signs	X	X	X	X	X
C-SSRS	X		X		X
MOCA, RBANS	X				
Neuropsychological Battery		X	X		X
Neuroimaging Assessments (fMRI)[f]		X[g]	X		X
Blood Draw for AGB101 level			X	X	X
Randomization		X			
Dispense Medication		X		X	
Administer Study Medication			X		X
Medication Compliance			X		X
Adverse Events	X	X	X	X	X[f]

[a] The ICF administered at the screening visit will address the blood (or buccal swab) for APOE and other screening procedures. If the study subject is eligible to enroll in the study, a separate ICF administered at the baseline visit will address the remainder of the study.

[b] Study procedures will take place on the last day of each 2-week treatment period.

[c] Medication is dispensed and other procedures obtained on the last day of the 4-week (-/+ 1 week) washout period.

[d] In the case of early termination, the Period 2 procedures will be performed.

[e] Visit takes place on the last day of the study period which may be a minimum of 14 days and a maximum of 21 days.

[f] Prior to each fMRI scan, subjects will undergo MRI safety screening.

[g] Subjects will undergo mock scanner training. At subsequent visits, the subject may be excused from mock scanner training.

[h] Screening activities may be split into 2 or more visits as needed (i.e. separate visit for APOE testing only and subsequent screening visit(s) for other screening procedures). Informed Consent #1 will be obtained prior to all activities at the first screening visit. With the exception of APOE genetic testing, all other screening procedures must be repeated if greater than 3 months elapse from the date of the first screening procedure.

[i] Start of Period 2 will be a minimum of 21 days and a maximum of 35 days post the date of the last dose of Period 1. (i.e. Washout period of 4 weeks, -/+ 1 week window permitted for scheduling purposes)

[f] Telephone call to subject at 4 weeks post last dose in Period 2 (-/+ 1 week) to assess for AEs and AE outcomes.

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