Network-Level Mechanisms for Preclinical Alzheimer's Disease Development

Principal Investigator: Shi-Jiang Li, PhD (Department of Biophysics)

Co-investigator: Piero G. Antuono, MD (Department of Neurology)

Address
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI 53226

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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 $\widehat{\boldsymbol{HFC}}^{+}\boldsymbol{(s,g,t)} \equiv \boldsymbol{Res(s,g,t)}$. 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* $\widehat{\boldsymbol{HFC}}^{+}\boldsymbol{(s,g,t)}$ 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