**Study Title:** Mechanisms of Antidepressant Non-Response in Late-Life Depression
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**Data analysis plan.** *Overview:* The proposed trial will enroll 130 patients aged 60-90 years and randomize them to open label treatment with escitalopram (n=60) vs. a double-blind escitalopram-placebo trial (n=70, with n=60 to escitalopram and n=10 to placebo). Hence, participants in the double-blind condition will not know whether they are receiving drug or placebo. Patients will vary in their baseline severity of ED and WMH burden, which will be measured using the Stroop Color-Word Test and the Fazekas modified Coffey Rating Scale of anatomical MRI images, respectively. Specific Aim 1 focuses on determining whether differences in expectancy and depression outcomes found over time between the two randomized conditions are ***moderated*** by baseline ED and WMH measures (i.e., whether more severe ED and/or WMH are associated with smaller differences between the open and placebo-controlled conditions). Specific Aim 2 investigates whether poor antidepressant outcome (regardless of randomized condition) is associated with more severe baseline ED and WMH burden, and whether this association is ***mediated*** by decreased patient expectancy. In the Exploratory Aim, we are interested in using DTI to explore with greater anatomical precision the white matter damage associated with smaller differences in patient expectancy and antidepressant outcome between the open and placebo-controlled conditions.

$$\textbf{MODEL 1: } Y_{ij} = \beta_0 + \beta_1 y_{i0} + \beta_2 g(t_j) + \beta_3 I_i^{TRT} + \beta_4 ED/WMH_i + \beta_5 g(t_j) * I_i^{TRT} + \beta_6 g(t_j) * ED/WMH_i + \beta_7 I_i^{TRT} * ED/WMH_i + \beta_8 g(t_j) * I_i^{TRT} * ED/WMH_i + \beta_9 X_i + b_i + \varepsilon_{ij}$$

Before the specific statistical techniques are applied, we will examine all variables at all time points for illegitimate values, outliers, and inconsistencies. The distribution of demographic variables, rater-administered and self-report measures will be examined and described in terms of means, standard deviations, minima, and maxima for continuous variables, and proportions for categorical variables. Missing data: We will make every effort to obtain all data. The longitudinal mixed modeling described below imputes unobserved time points using observed data from the individual combined with the overall trend. Inferences are valid if missing data are missing at random (MAR). MAR assumptions cannot be verified. A 2010 national expert panel[134] recommended sensitivity analyses for the impact of missing data via pattern mixture models,[135] which we will conduct (e.g., by investigating robustness of results to perturbations of assumed values for missing data within clinically plausibly ranges).

*Analyses for Specific Aim 1:* Hypothesis: Greater ED and WMH will be associated with smaller differences in patient expectancy and antidepressant outcome between the open and placebo-controlled conditions. All tests will be two-sided: α=0.05, intent-to-treat, with data included for all patients with ≥1 post-baseline assessment. The primary outcome measure of expectancy (items 2 and 4 of the CES) and antidepressant outcome (24-item HRSD) will be collected at baseline and at weeks 1 through 8. Analysis for expectancy will include all randomized individuals (n=130), analysis for HRSD will drop the n=10 individuals who receive placebo to strictly compare the antidepressant drug outcome of open label vs. double-blind. The hypothesis is addressed by testing the ***3-way interaction*** ($\beta_8$ in Model 1 below*)* between baseline ED/WMH, time, and treatment condition. 'ED/WMH' denotes our intention to analyze the effects of ED and WMH individually as well as in combination. The longitudinal mixed effect model (Model 1) will be used where $Y_{ij}$ is the expectancy or HRSD score for subject *i* at $t_j$ week, $t_j$=1,2,…,8, $y_{i0}$ is expectancy or HRSD at baseline, $I_i^{TRT}$ is the indicator variable for randomized condition (open label vs. double-blind), $ED/WMH_i$ is the baseline measure of ED/WMH (see section 3.3.B.6), $X_i$ includes the randomization stratification variable (i.e., age)[136] and any baseline covariates exhibiting imbalance after randomization, $b_i$ is a random intercept to account for repeated measures across time within patient, and $g(t_j)$ is a general function of time to allow for non-linear time trends. We will determine the best way to model $g(t_j)$ by comparing BIC fit criteria across models including linear, cubic splines, or categorical (i.e., dummy variable) time effects. Model 1 will be fit using PROC GLIMIX in SAS. A significant interaction of $g(t_j) * I_i^{TRT} * ED/WMH_i$ (corresponding to rejecting the null hypothesis that $\beta_8 = 0$) indicates that the effect of randomized condition on the outcome over time is different by baseline ED/WMH. Because this interaction effect is a function of continuous time and ED/WMH, interpretation will be facilitated by forming contrasts at different fixed values of time (e.g., early vs. late, week 1 vs. week 8) and ED/WMH severity (low vs. moderate vs. high defined by tertiles of baseline ED/WMH distributions). Model 1 will also be used to analyze secondary measures of expectancy (QIDS-SR Expectancy Version) and to test moderator effects of secondary measures of ED and WMH (see sections 3.3.B.5-6.)

*Analyses for Specific Aim 2:* Hypothesis: ED and WMH will be associated with decreased change in depressive symptoms, and this association will be mediated by a decreased contribution of expectancy to antidepressant outcome. The first part of this hypothesis will be tested using a longitudinal mixed effect for HRSD scores as in Model 1 but only including predictors that do not involve randomized condition, i.e. $\beta_0$, $\beta_1$, $\beta_2$, $\beta_4$, $\beta_6$, $\beta_9$. The tests of interest are the main effect for ED/WMH and its interaction with time (i.e. $\beta_4$ and $\beta_6$). After having established an overall effect of ED and/or WMH on depression outcome (i.e., HRSD), we will test

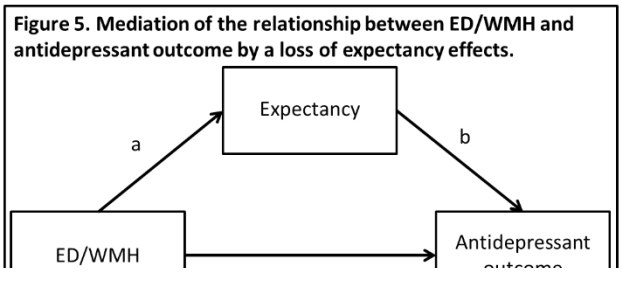

**Figure 5. Mediation of the relationship between ED/WMH and antidepressant outcome by a loss of expectancy effects.**

expectancy as a mediator of the relationships (see Figure 5). To be a mediator we must establish that expectancy is affected by ED/WMH and that it influences antidepressant outcome controlling for ED/WMH.[137] In order to ensure time order of the mediator (expectancy) occurring prior to the depression outcome (HRSD), we will use early change in expectancy (i.e., from baseline to week 1) as the primary measure for the mediator.[138] A model with week 1 expectancy as the outcome and ED/WMH as the primary predictor, controlling for baseline covariates, will be used to estimate path a in Figure 5. To estimate path b in Figure 5, the same longitudinal mixed effect model described above with the addition of week 1 expectancy as a predictor will be used. The mediation effect is estimated by taking the product of the estimates for a and b and using the bootstrap method to obtain standard errors, confidence intervals, and the test for statistical significance.[139] Recent developments in mediation analysis suggest the need for sensitivity analyses to explore potential impact of unmeasured confounders.[140] We will perform these sensitivity analyses with the new mediation R package that provides a range of plausible results that may have been obtained under different assumptions about unmeasured confounders.[141]

*Analyses for Exploratory Aim:* As described above, DTI data for each subject will yield a voxel-wise map of FA values across the entire brain. Substituting these FA values for ED/WMH in Model 1, we will assess at each voxel whether the FA values differ significantly by randomized condition on expectancy or antidepressant outcome over time. Although Bonferroni correction could be employed to correct for multiple statistical comparisons, it tends to be overly conservative since the sets of FA correlate with one another. Alternatively, we will use statistical analyses based on the theory of Gaussian random fields (GRFs) defined across the entire brain to account for correlations across voxels and to calculate corrected p-values.[142] In addition to GRF-corrected maps, we will also apply the theory of False Discovery Rate (FDR) to control for multiple comparisons while permitting discovery of 5% false positives.[143] Because the method for FDR permits a specified percent of false positive, the method for FDR is statistically more powerful in detecting significant differences in the brain. Our method also allows us to include the use of covariates such as age, sex, and IQ when calculating corrected P-values. Next, we will identify the neural pathways associated with voxels having significant $g(t_j)^* I_i^{TRT} * FA_i$ interactions by coregistering the template DTI brain to the DTI atlas described above. Finally, we will assess the integrity of the frontostriatal and frontolimbic tracts by performing tractography from PFC regions to striatal and limbic areas and assessing whether the significant voxels lie along the fiber tracts.